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7256 C*

Protective Effect of Iron and Copper in Infectious Anemia of the Rat.†

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Pure iron, alone or in combination with a small optimum dose of copper or other catalytic metals, or combined with ultraviolet rays, is able to prevent and cure nutritional anemia in the weanling rat when fed upon a diet of whole milk for a period of 6-9 weeks.[1, 2] A similar preventive effect of both iron and copper has recently been advanced by Perla and Marmorston-Gottesman[3] against the anemia of the splenectomized rat produced by Bartonella infection. It was further suggested by these investigators that the so-called nutritional anemia of the white rat may be complicated or influenced by a spontaneous infection of the animal with Bartonella.

Perla observed that the addition of copper, either when fed or injected, to an adequate diet for a period of 10 days prior to splenectomy and 1 month thereafter protected 75% of their animals against the anemia, while iron, under similar conditions, protected against anemia in 50% of the animals. It was stated that copper is essential with iron in hemoglobin regeneration in rats suffering from nutritional anemia and also that an excess of copper plays a rôle in the mechanism of resistance to *Bartonella muris* anemia in the rat after splenectomy.

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* P represents a preliminary, C a complete manuscript.

† We wish to thank the American Academy of Arts and Sciences for a grant from their Permanent Science Fund for this and the following study.

[1] Beard, H. H., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 89.

[2] Beard, H. H., Johnson, A. G., and Andes, E. J., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 23.

[3] Perla, D., and Marmorston-Gottesman, *J. Exp. Med.*, 1932, **56**, 783.

The following work was undertaken to compare the effect of both iron and copper in this infectious anemia with their action in nutritional anemia.

Eighty animals were kept in large galvanized iron cages recently painted with aluminum paint and fed with Purina Dog Chow‡ which represents the standard food for our stock animals in this study. Groups of 10 animals were usually kept together in one cage so as better to expose them to the possibility of infection. Four cages with 20 adult and 20 young rats served as control. In order to avoid mistakes due to irregular intake of metals by the animals, the colloidal copper and iron preparations were administered by intraperitoneal injection. To omit trauma the injections were given every fifth day commencing 10 days previous to splenectomy and subsequent thereto for one month. 2.5 mg. of the iron were given to the first group; 2.5 mg. of iron-copper solution were given to the second group; 0.25 mg. of the copper solution were given to the third group which corresponds to a daily dose of 0.5 mg. iron and 0.05 mg. copper, doses which proved optimal in the treatment of nutritional anemia. These doses are one-half those used by Perla. One group of 10 rats was exposed daily for 30 minutes to the rays of the General Electric Sunlight Mazda Lamp, Type S-I, (at a distance of 4 feet) which has been used in previous experiments.[2]

The animals were splenectomized with the usual technic and red cell counts and blood smears were examined every third day. The splenectomized animals were under observation for 2 months when this report was made. The results are shown in Table I.

It is clearly seen that the animals on Purina Dog Chow (groups A and B) containing sufficient iron and copper, were heavily in-

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‡ The composition of this product was as follows: meat, wheat germ meal, dried skim milk, cod liver oil, barley malt, blackstrap molasses, oat cereal, corn cereal, wheat bran and iodized salt. Analysis: Protein 20.0%; fat 3.0%; fibre 6.0%; nitrogen-free-extract 50.0%; carbohydrates 56.0%; ash 6.0%; having a caloric value of 1,500 calories per pound. This diet contains ample copper and iron. (Popular Bull. No. 4, Research Department, Purina Mills, St. Louis.)

TABLE I.

Group	Supplement	No. of animals used	No. of animals infected with *Bartonella M.*			No. of animals protected against infection
			Died	Recovered	Total	
A	Normal adult rats fed on Purina Dog Chow	20	6	13	19	1
B	Normal young rats fed on Purina Dog Chow	20	18	1	19	1
C	Intraperitoneal administration of Copper in form of CuS (Kupferdiasporal)	10	3	4	7	3
D	Intraperitoneal administration of Iron in form of colloidal highly dispersed ferrous hydroxide (Eisendiasporal)	10	7	3	10	0
E	Intraperitoneal administration of colloidal-highly dispersed Iron-Copper solution	10	5	5	10	0
F	Administration of Ultraviolet Rays	10	8	1	9	1

fected with Bartonella, which was more fatal in the young than it was in the adult animals. In group C, receiving copper alone only 3 out of 10 rats were protected from the anemia. These results resemble somewhat those of Perla, who, however, observed a much better protective effect with copper. Intraperitoneal injection of iron, iron and copper, and iron plus ultraviolet rays, to groups D, E, and F, respectively, did not afford protection to any of the animals.

We conclude from these observations that iron and copper, in doses which prevent and cure nutritional anemia in the albino rat, do not protect the animal against the anemia of Bartonella infection following splenectomy.

7257 C

Pathological Changes in Liver and Spleen in Nutritional Anemia in Rat.

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Pure milk fed to young rats produces severe anemia which finally results in death in the majority of cases. In our studies of the pathological changes which take place during the course of nutritional anemia in the albino rat, the findings in the liver and spleen seem to have considerable value in the differential diagnosis of this type of anemia as contrasted with the infectious type caused by *Bartonella muris*. All our examined animals showed a severe degree of anemia with an erythrocyte count of less than 3 million per c.mm. and a Hb content of less than 4 gm. per 100 cc. Most of the animals died spontaneously, while 5 were killed during the course of the disease. The spontaneous death of the anemic animals was consistently preceded by a rapid loss of weight during the last days of life. The liver and spleen were weighed and fixed with the other organs in Zenker, alcohol and formalin. Slides for microscopic study were stained with hematoxylin-eosin, van Gieson, Mallory, Bielschovsky, Sudan III and Turnbull's Iron stain.

Changes in the Liver. Macroscopically, the changes were not marked. The size and weight were slightly decreased, the surface smooth and pale, the consistency soft. The cut surface showed a slight yellowish brown mottling and occasional small petechial hemorrhages.

The microscopic changes were most pronounced in those animals which died spontaneously from anemia. The central veins and the capillaries in the center of the lobule were greatly distended and filled with an edematous fluid. In the center of the liver lobule the cells were atrophied; those near the center were swollen and showed a marked vacuolization of the plasma. The majority of these vacuoles proved to be fat droplets when stained with Sudan III. They completely filled the cells surrounding the atrophic areas in the center of the lobule and were present even in livers in which atrophy of the central part of the lobules was just in the beginning stage. The periportal zones showed marked hypertrophy of the cells, with no fat, and an increase in the size of the cell and of the nucleus. Most of the cells contained 2 or more nuclei. Van Gieson, Mallory and Bielschovsky stains showed an early increase of gitter fibres in the center of the lobules which also was present before the atrophy of the center became manifested. The Kupffer star cells and the other endothelial cells were small, the nucleus being pyknotic in many cases. Iron pigment was absent. The periportal collections of small round cells which are so common in the normal rat and guinea pig were entirely absent.

The picture of nutritional anemia resembles that of chronic passive congestion with secondary atrophy of the liver parenchyma. The fatty changes, however, are much more pronounced than those

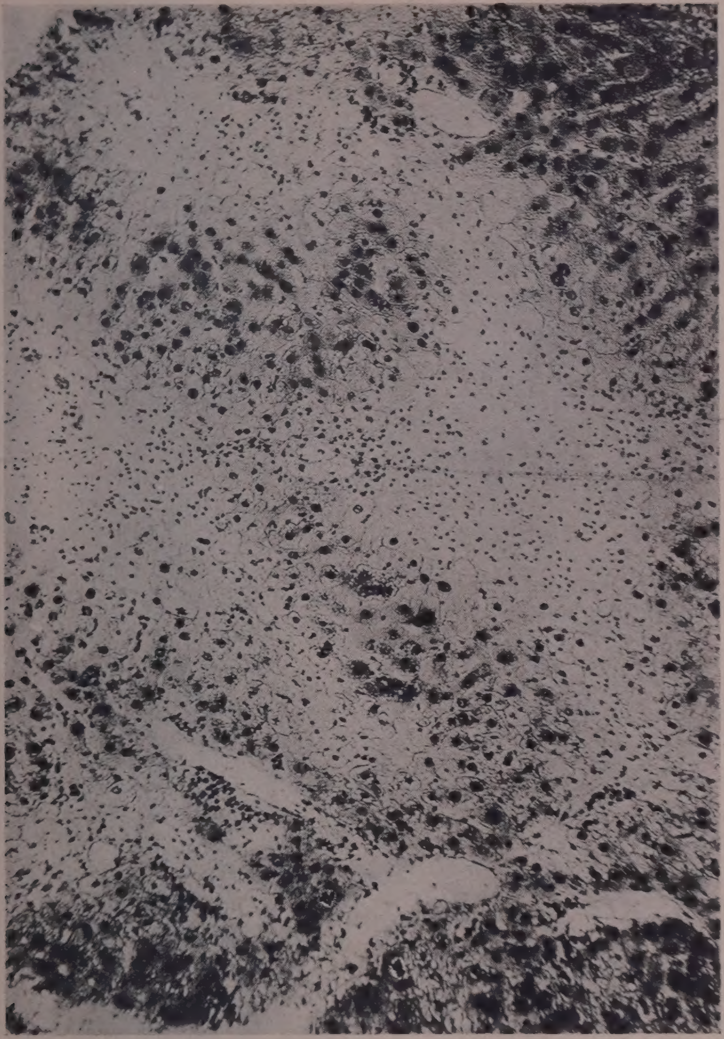

FIG. 1.
Changes in the liver.

found in chronic congestion. The absence of hemosiderin in the endothelial cells and the presence of fluid in the capillaries are more or less typical characteristics of the changes found in the liver during nutritional anemia.

Changes in the Spleen. Macroscopically, no distinct gross path-

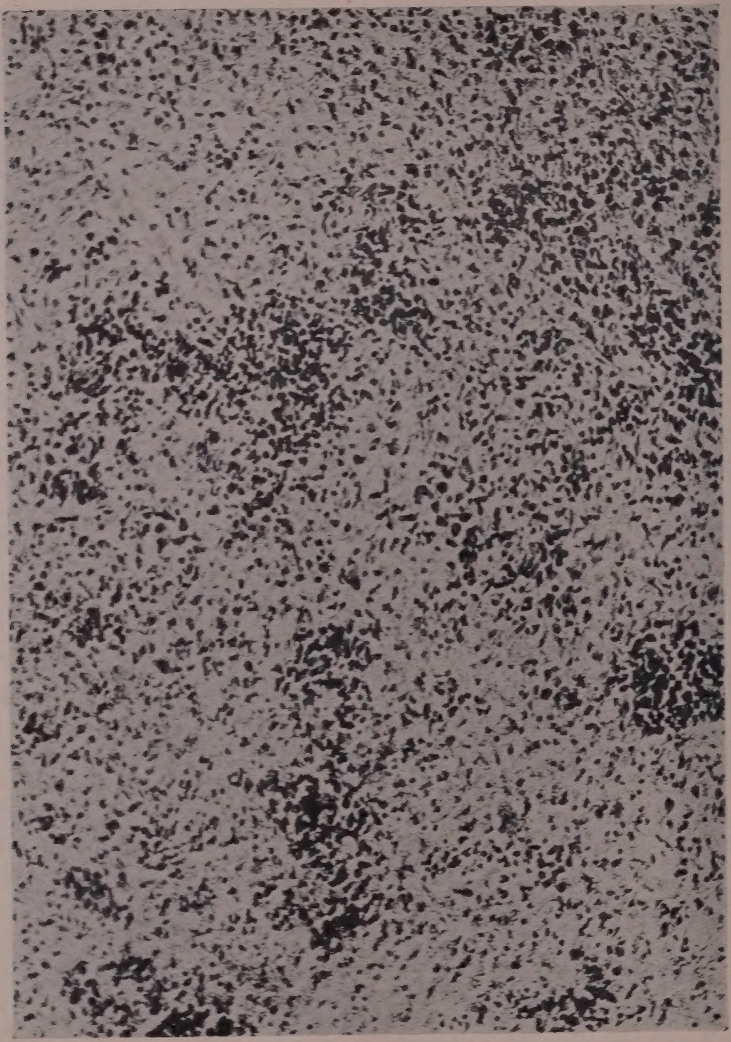

FIG. 2.
Changes in the spleen.

ological changes were present. The spleen was small, pale and soft.

Microscopic examination showed fewer and smaller lymph follicles than those found in the normal organ. There was also a marked decrease in the size of the perifollicular zone of the pulpar cells. The sinuses were dilated and filled with a pink staining fluid.

The pulpar cells showed all the signs of degeneration including complete necrosis with karyorrhexis and pyknosis. The nuclei of the megakaryocytes also showed pyknosis. Iron pigment was absent. In the spleen also, the dissimilarity of the histological picture to that of infectious anemia, with all the signs of splenic hyperactivity, is marked.

Reviewing the changes which take place in the liver and spleen of animals dying from nutritional anemia or killed in the later stage of the disease, we note (1) the absence of any signs of hematopoiesis or hematocataresis, and (2) the presence of severe atrophic disturbances of the cells, associated with signs of failing circulation (chronic heart failure).

7258 P

Use of Living Chick Embryos in the Propagation of B. leprae.

R. A. HOLT. (Introduced by C. W. Duval.)

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Because of our failure to obtain *in vitro* multiplication of *B. leprae* in minced chick embryo media,[1] it occurred to us that living chick embryos might afford a more suitable nutritive since the studies of Duval[2] indicate that *B. leprae* is unable to cleave whole protein and that the split products (amino acids) are essential for its multiplication. This hypothesis is substantiated by the observations that the bacilli apparently enter and colonize within the living host cells without destroying them and, as intracellular sojourners, utilize the nutrients intended for the latter; that artificial culture media containing the end products of protein digestion afford a favorable foodstuff for the initial cultivation of *B. leprae;* and that in removed bits of leprous tissue in which autolysis has taken place, the Hansen rods continue to proliferate so long as there are amino-acids present, while in subsequent transplants from these bits of tissue, growth becomes more feeble as the protein split products decrease in amount. Further, the living chick embryo was thought a desirable medium since it is known that there is a large supply of split protein accessible to its growing cells. The question of susceptibility of the living chick embryo to leprosy and the adaptation of *B. leprae* to this environment was considered. In this connection, it is realized that very few lower animals are susceptible to *B. leprae* propagation.

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[1] Holt, R. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 567.

[2] Duval, C. W., *J. Exp. Med.*, 1910, **12**, 649.

The object of this study was to determine the time required for the growth and multiplication of *B. leprae* in the living chick embryonic tissue, the *modus operandi* of the invasion and the effects of colonization upon the living cells. That part of the study which deals with the time required for *in vivo* cultivation of *B. leprae* will be considered in this paper.

The method of Goodpasture[3] was utilized. In brief, fertile hens eggs were allowed to incubate in ordinary laboratory incubator at 38°-40°C. in the presence of moisture. The eggs were candled after incubation for 10 to 14 days. If development of the embryos had occurred, square windows were cut in the shells and shell membranes, taking care to avoid injury to all other membranes. Through these windows inoculations were made upon the chorio-allantoic membranes. Coverslips were then sealed over the holes in the shells with sterile paraffin-vaseline mixture. The reactions of the chorio-allantoic membranes and activities of the embryos were conveniently observed through the windows. Near the end of embryonic development, the embryos, membranes and egg substances were fixed *in situ* with Zenker's fluid or formalin, sectioned and stained with hematoxylin-eosin and by the Ziehl-Neelsen method for the demonstration of acid-fast bacilli.

The materials used for inoculations were emulsions of human leprous nodules in sterile Ringer's solution. The leprous tissue was received from Dr. O. E. Denney, Director, National Leprosorium, Carville, Louisiana. Some of these tissues were contaminated, while others were not. If contaminated, the emulsions were treated for one hour with 3% sodium hydroxide and then washed 3 times in Ringer's solution.

Macroscopic observations through the windows during the development of the embryos repeatedly revealed very definite inflammatory responses in the allantoic membranes as evidenced by a marked distension of the vessels and its distinct thickness and opaqueness.

Microscopic examinations of sections of the embryos, membranes and egg substances that were stained by Ziehl-Neelsen method gave no evidence of the multiplication of *B. leprae*. The acid-fast bacilli and "globi" observed were considered to be those placed there in the original inoculations since, by comparison with the index inoculum-slides, there was no increase in number, no loosening of the "globi" and no change in morphology. Furthermore, the host reaction as noted in the hematoxylin-eosin stained sections was that of a sub-acute and chronic inflammation such as occurs about an inert foreign body. The tissue reaction was characterized by lymphoid, plasma and fibroblastic cells. Neutrophiles were conspicuous by their absence. Endothelial cells were present in and around the tissue particles of the inoculum. However, there was no evidence that these cells had phagocytized the *B. leprae* or that the latter had invaded them.

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[3] Goodpasture, E. W., *So. Med. J.*, 1933, **26**, 418.

These results indicate to us that the multiplication of *B. leprae* does not occur *in vivo* in chick embryos within 7 to 11 days. Experiments are in progress in which successive transplants from one chick embryo to another are made. We hope to determine in this way if a longer period of incubation will result in *B. leprae* multiplication, host-cell invasion and intracellular colonization. In conclusion, we feel that living chick embryo may be satisfactory for the study of the *in vivo* behavior of *B. leprae*.

7259 C

Morphological Resemblance of the Rod Shaped Pigment of the Chick Retina to B. Leprae.

R. A. HOLT. (Introduced by C. W. Duval.)

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During recent attempts to cultivate *B. leprae* in embryonic chick tissues,[1] an interesting observation was made on the close morphological resemblance of this microorganism in freshly excised human leprous tissue to the rod shaped pigment of the chick retina. This observation is considered worthy of note because of the possibility of confusing these bacilli and pigment rods when employing embryonic tissue as a culture medium. Furthermore, as Rivers[2] points out, the presence of the pigment rods may lead to confusion when smears are made to determine the sterility of the cultures. For this reason, he suggests removing the eyes of the embryo before making use of the tissue as a medium. We, however, prefer to include as much of the highly specialized tissues as possible and, therefore, make use of the entire embryo.

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[1] Holt, R. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 567, 643.

[2] Rivers, T. M., *J. Exp. Med.*, 1931, **54**, 453.

The rod-shaped pigments are found within the inner surface of the hexagonal epithelial cells of the retina, this layer being considered the outer layer of the optic cup. They occur normally in tightly packed masses and usually with regularity. However, their arrangement can be readily disturbed by mechanical means; that is, the packets are easily broken by ordinary manipulation and their component rods are freely scattered when embryos are either minced or sectioned. When this dispersion occurs, these pigment rods and packets can be mistaken morphologically for those of *B. leprae* and the "globi" found in human leprous tissue. The individual pigment rods are 1-5μ in length, dark brown in color and resistant to the penetration of dyes. The distribution of the pigment within the rods is usually homogeneous, but sometimes it is irregular and simulates the chromatin "beading" so characteristic of acid-fast bacilli.

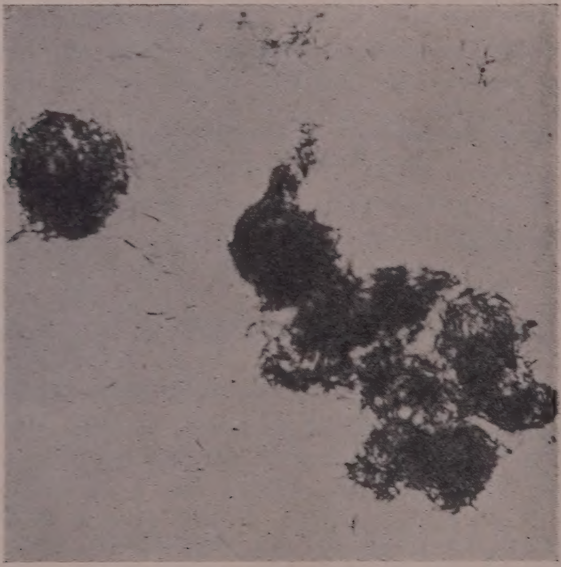

FIG. 1.
Smear preparation of chick embryonic tissue showing "globus" appearance of rod shaped pigment within the epithelial cells of the retina. A few individual rods are scattered while others are in small packets. Ziehl-Neelsen stain. × 1000.

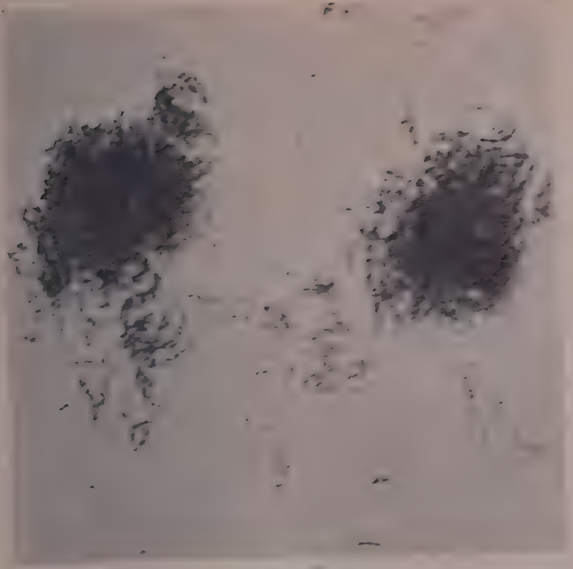

Fig. 1.

Smear preparation from human leprous nodule showing typical "globi". Some of the leprosy bacilli are seen free from the tightly packed masses while others occur in small clusters. Ziehl-Neelsen stain. × 1000.

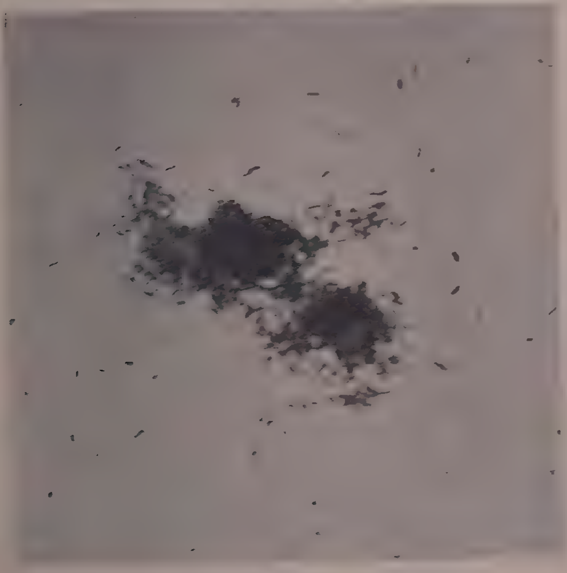

Fig. 2.

Mechanical dispersion of the rod-shaped pigment after rupture of the chick retinal epithelial cells. Ziehl-Neelsen stain. × 1000.

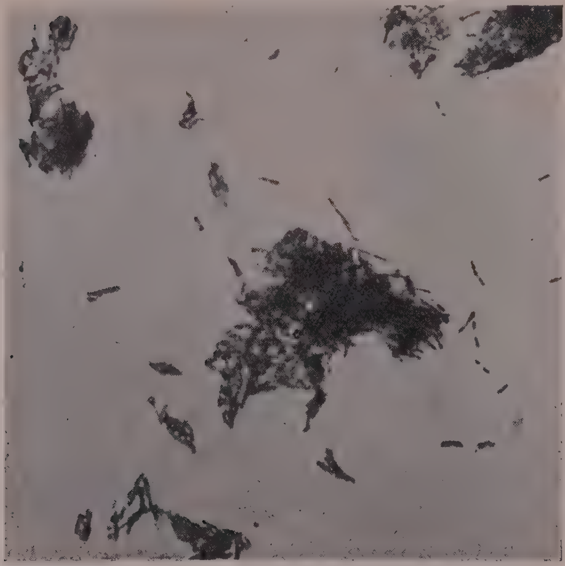

FIG. 4.
Mechanical dispersion of *B. leprae* after rupture of the characteristic leprous "globi". Ziehl-Neelsen stain. × 1000.

Differentiation of these pigments and bacilli can be made by tinctorial means. The best staining method for this purpose is that of Ziehl-Neelsen, since *B. leprae* is acid-fast while the pigment rods remain brown. The Gram and hemotoxylin-eosin stains can be used, but the contrast between the bacteria and pigments is not so definite and often the 2 are confused. Differentiation can further be made by fixing the material for examination to the slide and studying an unstained preparation. Here again, the normal color of the pigments is seen. The unstained *B. leprae,* in contrast, can only be observed when viewed in transmitted light, except for their chromatic "granules" which appear as small, round, blue-black bodies.

In conclusion, we wish to point out the close morphological resemblance of the rod-shaped pigments of the chick retina and their "globus" arrangement to the *B. leprae* and "globi" found in leprous tissue. Emphasis is placed upon the possibility of confusing these normal retinal structures with the *in vitro* multiplication of the leprosy bacillus in a minced chick embryo culture medium.

7260 P

Effect of Carbon Arc Radiation on the Cardiac Output of Hypertensives.

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Irradiation with the carbon arc lowers the blood pressure of man, particularly in those in whom it is abnormally high.[1] To produce a lowering it is necessary to give a dose of radiation which causes a marked and lasting peripheral vasodilatation.

As part of a study of the influence of carbon arc radiation, it was decided to investigate the cardiac output by the Grollman[2] method.

A total of 16 irradiations was given to a group of 5 normal controls (medical students and instructors). In every case but one the exposure was followed by an increase in the cardiac output, which reached its peak as a rule on the second or third day, and then returned to normal by the fifth or sixth day. The increase ranged from 12 to 31% and averaged 20%. This is in agreement with the work of Lindhard,[3] who found a significant increase in 4 out of 7 subjects after ultraviolet irradiation. The blood pressures for the control group showed an average drop of 6 mm. for the systolic and 8 mm. for the diastolic.

The hypertensive group, with basal blood pressures ranging from 191 to 128 mm. systolic, and 121 to 74 mm. diastolic, was made up of 9 patients with essential hypertension and one with interstitial nephritis. They were given a total of 28 irradiations, and in every case but one there was a fall in blood pressure, the average drop being 19 mm. for the systolic and 10 mm. for the diastolic. The cardiac output increased in 16 instances by an average of 34%, decreased in 8 by an average of 22%, and showed no change in 4.

The figure shows changes in the systolic blood pressure and cardiac output for both groups for several days following a single irradiation. Each point on the curves is determined by the arithmetical average of the changes occuring on that particular day, and is made up from data on all the separate experiments. The greatest response in the hypertensive group occurs on the first day following an irra-

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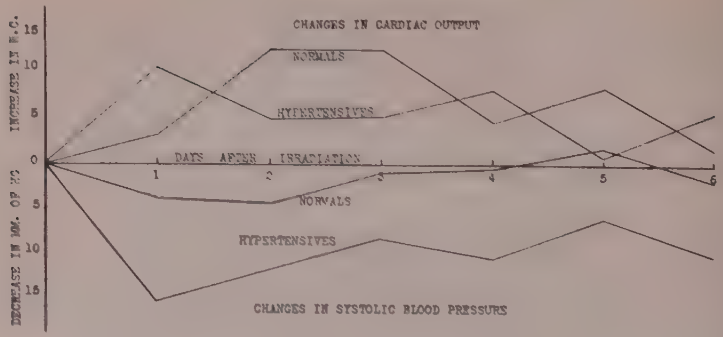
[1] Laurens, H., The Physiological Effects of Radiant Energy, 1933, 168.

[2] Grollman, Arthur, *Am. J. Physiol.*, 1929, **88**, 432.

[3] Lindhard, J., *Skand. Arch. f. Physiol.*, 1913, **30**, 73.



diation, while in the normals it is not manifest until the second or third day. In both types of individuals the first and foremost reaction to the irradiation is a widespread peripheral vasodilatation. In normal individuals this is compensated almost entirely by an increase in the cardiac output, the blood pressure remaining practically unchanged. In the hypertensives, on the other hand, there is nearly always a significant depression of the blood pressure, but the cardiac output, as above stated, may in individual cases increase, decrease, or remain unchanged. The prevailing response, however, is an increase, as in the normals, as shown by both its more frequent occurrence and its greater degree of magnitude.

These changes are quite temporary as a rule, and do not outlast the erythema to any extent. In a few cases the depression of blood pressure in the hypertensives has persisted for 10 to 12 days following the last irradiation.

7261 P

Effect of Light and of Darkness on Thyroid Gland.

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That darkness or the absence of ultraviolet radiation, particularly of the antirachitic portion, leads to an hyperplasia of the thyroid gland has been suggested by some investigators[1] and denied by others.[2] A critical analysis of these results, however, reveals many discrepancies and contradictions.

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[1] See review by Levine, H., and Remington, R. E., *J. Chem. Ed.*, 1933, **10**, 649.

The present study extended from November, 1932, through July, 1933, and involved the use of 140 albino rats. Of this number, 40 were offspring of stock animals bred and kept in the rat room illuminated by diffuse daylight filtered through window glass. The remainder were offspring of rats kept and bred in a dark room, the only radiation coming from a ruby lamp used for a brief period daily while feeding, cleaning, or weighing. The animals were grouped at weaning (21-28 days), weighed weekly, and kept under the various experimental conditions until they were from 12 to 16 weeks old. At the end of each 4-week period, representative animals from each group were sacrificed and the thyroids removed for histological examination. The experiments were divided into 2 series: (1) in which the rats were fed a balanced stock ration, Bal-Ra, rich in iodine; (2) in which the rats were fed bread and fresh milk, a diet relatively poor in iodine. In the first series, one-half of the animals born in light were allowed to remain in the "light room" and groups exposed for 15 minutes every other day at a distance of 1 meter from a flaming carbon arc, burning "Sunshine" carbons (total energy value of each exposure = 6.51 gm. cal. per sq. cm.; ultraviolet = 0.273 gm. cal. per sq. cm.) and to sunlight from 10 A. M. to 2 P. M. daily. The remaining 20 rats were placed in the dark room and kept there except when they were being irradiated. The same number of animals (40) born in darkness was divided so that half remained in the dark and the rest in the "light room" and were irradiated as above. The second series consisted entirely of animals born in the dark and was divided in a similar manner, except that in this series additional groups were exposed to the radiation from a quartz mercury vapor arc for 15 minutes every other day at 1 meter (total energy value of each exposure = 0.591 gm. cal. per sq. cm.; ultraviolet = 0.041 gm. cal. per sq. cm.) and to sunlight (4 hours daily) through window glass transmitting no wave lengths shorter than 310 mμ and under Corex purple glass, which transmits only ultraviolet radiation. Appropriate control groups were maintained throughout.

No significant differences could be detected in any of the experiments with respect to the body growth, size or histological picture of the thyroid gland. With few exceptions, all of the rats fed the stock diet in Series 1 showed non-hyperplastic thyroids irrespective of light environment. Likewise with few exceptions, the animals in Series 2 fed bread and milk showed various degrees of hyperplasia, which condition was not corrected or perceptibly modified by any of our experimental procedures. Our experiments fail to confirm the suggestion that darkness leads to thyroid hyperplasia or that antirachitic ultraviolet radiation is essential for the maintenance of proper thyroid development.

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[2] Von Fellenberg, T., *Biochem. Z.*, 1931, **231**, 205; McCarrison, R., *Indian J. Med. Res.*, 1932, **20**, 359; Kraus, W. E., and Monroe, C. F., *J. Biol. Chem.*, 1930, **89**, 581.

Minnesota Section.

University of Minnesota, February 21, 1934.

7262 P

A Dehydrated Bacterial Agglutination Antigen (Bang's Disease).*†

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This is a preliminary report on the use of a dehydrated bacterial antigen for agglutination testing. The studies reported are confined to *Bact. abortus* antigen used in the diagnosis of Bang's disease of cattle.

Preparations of bacterial agglutination antigens for use in macroscopic methods of agglutination testing for disease have universally been made by suspending the bacteria in salt solution. The early workers[1] in this field considered the salt solution as essential for the agglutination phenomenon. This conclusion is known now[2, 3] to be erroneous.

We are studying a method of agglutination testing in which a washed, dehydrated antigen preparation is mixed in measured quantities with fixed amounts of agglutination serum. The bacterial suspension, in distilled water, is evaporated to dryness in petri dishes in the incubator at 37.5°C. A film results which is transparent, amber colored and quite brittle. The antigen is kept in the dehydrated form until needed for use.

A satisfactory method of conveying the dehydrated antigen to the agglutinating serum in the necessary minute measured amounts has been effected by resuspending the antigen in distilled water. By suspending weighed quantities of the dehydrated antigen in definite amounts of water and pipetting this suspension, it is possible to measure precise amounts of the antigen onto a suitable glass plate. The water is again removed by evaporation before an electric fan. The antigen adheres to the glass plate.

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* Paper No. 1257 of the Journal Series of the Minnesota Agricultural Experiment Station.

† These studies were made possible by a grant from the Bureau of Animal Industry, United States Department of Agriculture. They were aided somewhat by assistance provided through the Civil Works Administration of the United States Government.

[1] Bordet, Jules, *Ann. Inst. Pasteur*, 1899, **13**, 225.

[2] Donham, C. R., and Fitch, C. P., *J. Inf. Dis.*, 1933, **53**, 98.

[3] Unpublished data.

Measured amounts of agglutinating serum are mixed with the dried antigen. This can be accomplished conveniently by stirring with an ordinary wooden toothpick. Macroscopic agglutination takes place rapidly, within one to several minutes, when the serum contains specific agglutinins.

It is possible to mix the serum and antigen in varying proportions and thus obtain a system of dilutions which gives agglutination titres comparable to those obtained by other methods of agglutination testing. We are not prepared to recommend definite proportions. Further studies are necessary. However, sufficient data are available to justify the following statements: The dilution system can be arranged so that the titres obtained with dehydrated antigen essentially agree with those obtained by the test-tube and rapid methods in testing most serums. There are, however, occasional serums which do not show agreement in titres when tested by the different methods.

So far the disagreement has always been in one direction, namely some serums cause agglutination of dehydrated antigen that do not cause it when tested with aqueous antigen preparations. Similarly some serums show much higher titres when tested with dehydrated antigen. Testing with dehydrated *Bact. abortus* antigen differentiates between some serums which have appeared to be identical serologically, when tested by the test-tube or rapid methods.

It remains to be shown whether the titres obtained with dehydrated antigen are more or less instructive than those obtained with aqueous preparations of the antigen.

We have accumulated a group of cattle that had, at the time of purchase, positive titres when tested with dehydrated antigen and either entirely negative or suspicious titres by the other methods. Some of these have terminated their pregnancies either by abortion or normal parturition and *Bact. abortus* has been isolated from them. Such animals have subsequently developed definitely positive agglutination titres when tested with aqueous preparations of the antigen. These serums showed positive agglutination titres when tested with dehydrated antigen earlier in the course of the disease than they did when tested with aqueous preparations of the antigen.

Other possible uses of dehydrated antigen which are being studied include: 1. Its use for preparing test fluids for the rapid and test-tube methods of agglutination testing, chiefly for standardizing the bacterial concentration and as a method of preservation over long periods of time. 2. Its use as a product for diagnosing Bang's disease by means of allergy.

7263 P

Localized Pain Accompanying Faradic Excitation of the Stomach and Duodenum.

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From the Departments of Anatomy and Radiology, University of Minnesota.

The following observations, hitherto unreported, were made during the course of experiments designed to test the reaction of the human gall bladder to faradic stimulation of the gastro-intestinal tract.[1]

Eleven medical students cooperated in this undertaking, several of them submitting to repeated experimentation. The device selected for stimulating the gut tract was a standard Rehfuss tube enclosing a copper wire soldered to the metal olive at the end of the tube. The other electrode consisted of a moist pad fastened to the arm of the subject. Through this circuit was sent an induction current that was almost intolerable when tested by the lips, but which was not unbearable when applied to the gut, causing sensations varying from barely perceptible, gnawing sensations, to heartburn and sharp colicky pain. With the aid of fluoroscope and barium meal, it was ascertained that when the olive was pushed against the wall of the stomach the current caused ring contraction of the gut and then increased peristalsis distal to that point. In each case the duration of the current was 10 seconds.

Figure 1 (left) records observations made upon one student on 4 different days. The circles indicate varying positions of the electrode in the gut (as determined by X-rays); the dots the site of the pain area on the abdominal wall. With the subject prone, excitation of the mid-pyloric stomach was localized at the lower middle or left epigastric regions; of the pyloric antrum, at the lower

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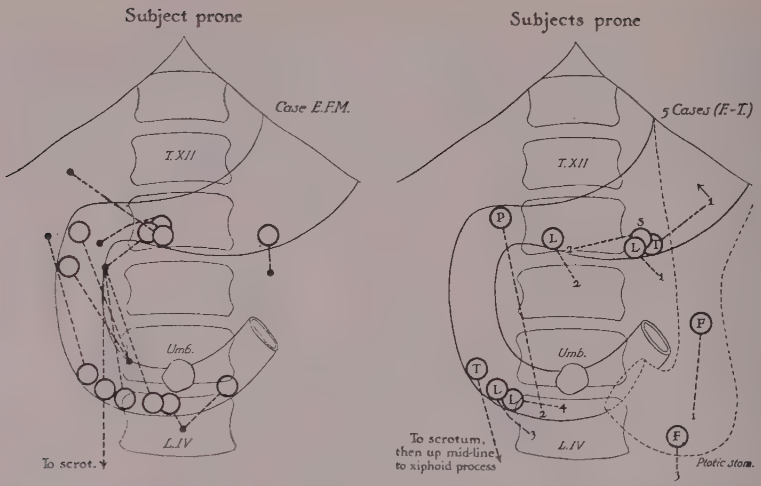
[1] Boyden, E. A., *Anat. Rec.*, 1933, **55** (Suppl.), 8.



middle or right epigastrium; of the pars superior (duod.) at the right umbilical or right supra-umbilical region; of the pars descendens at the right lower epigastric, the right umbilical, or the right scrotal region; of the pars inferior (duod.) at the mid-line just below the umbilicus.

Figure 1 (right side) records scattered observations on 5 other students. Case F (1-3) shows that in marked ptosis of the stomach, pain may be localized as low as the left umbilical zone. Similarly, other experiments have shown that when the subject is shifted from a prone to a side position or to his back, the site of pain frequently shifts with change in posture. Usually the pain is accompanied by varying degrees of abdominal rigidity.

Interpretation of these findings—namely as to whether the pain is entirely visceral, or is partly of anterior-peritoneal origin (according to the views of Morley[2]), or is referred, or is a combination of one or more of these—is being deferred, pending nerve-blocking experiments with novocaine.

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[2] Morley, John, Abdominal Pain, Edinburgh, 1931.

New York Meeting.

New York Academy of Medicine, March 21, 1934.

7264 P

Reaction of the Rabbit to Vaccine Virus.

LOUISE PEARCE, PAUL D. ROSAHN AND C. K. HU.

From the Laboratories of the Rockefeller Institute for Medical Research.

Little information is available regarding the clinical reaction of the rabbit to cutaneous inoculation of dermo-vaccine virus except as regards the local reaction at the site of injection. Furthermore, factors which presumably might affect the response as, for example, age, have not generally been considered. Our interest in the matter arose from the results obtained in the vaccination of the rabbits of a large breeding colony. The experiments are still in progress but enough has been learned to show that the clinical reaction of the rabbit to vaccination is extremely variable and that these variations are associated with a number of factors which concern the host.

Approximately 1800 rabbits were vaccinated in December, 1933, and January, 1934, with culture dermo-vaccine virus obtained through the kindness of Dr. T. M. Rivers. The injections were made intracutaneously with dilutions ranging from 1:50 to 1:40,000. Previous tests had shown that the virus was active in a titre of 1:100,000 to 1:1,000,000 injected intradermally. Certain outstanding results of the experiments may be briefly summarized as follows.

A typical local reaction developed in the adult non-immune stock of both sexes but it was much more severe in bucks than in does, and more pronounced in resting than in pregnant and nursing does. Generalized manifestations consisting in particular of a cutaneous maculo-papular eruption in areas remote from the injection site, as for example, the ears, were not infrequent. A lymph adenitis was also observed and an orchitis in male animals developed in many instances. There were a few cases of pronounced illness and prostration but only 3 fatalities.

The results were quite different in the younger stock, all of which were presumably susceptible. In the first place, a local reaction did not develop in a large proportion of these rabbits and the incidence of negative results was inversely proportional to age. Certain of these rabbits 1 to 7 days old, moreover, received much larger doses than the adults. Secondly, there was an unexpected high incidence of generalized clinical manifestations remote from the local injection site and surrounding shaved area. A maculo-papular or papular eruption highly variable in distribution, extent, and number of individual lesions, an adenitis, a conjunctivitis and other eye symptoms, an involvement of the nasal passages and sinuses, and intestinal disturbances were among the clinical features observed. Many of these animals were obviously ill and a considerable number of them died; others, however, recovered. Although generalized signs and symptoms occurred in animals in which a local vaccination reaction developed, it was noteworthy that the majority of the cases were in rabbits in which no local reaction of any kind had been detected. Another feature of interest was the occurrence of a relapse of generalized lesions in an occasional animal. In one case, no lesions could be found for 2 weeks and then there developed a fresh crop of typical papules. There were also a number of fatal cases in which no clinical signs of the disease had been observed prior to death but in which postmortem examination revealed typical vaccinal lesions of the viscera. Certain of these animals gave no reaction at the site of inoculation. In some instances the failure to develop a positive vaccinal reaction on the part of nursing young was associated with the immune state of the suckling doe.

Perhaps the most significant result obtained and certainly one which must be taken into account in considering the general question of vaccination, was in connection with latent infections. There were several instances in the very young stock in which the animals were completely negative clinically for periods of upwards of 2 months. There then developed typical generalized manifestations of varying degrees of severity.

There is strong evidence to believe that the failure to develop a primary lesion in certain age groups is due to a natural and transitory refractoriness in the host, and that when this refractory period is passed, the virus which has been harbored by the animal is operative in the production of generalized lesions. Thus certain aspects of these experiments lend support to the concept of a host acting as the carrier of a disease producing agent to which it is non-immune.

7265 C

Effect of Heavy Water (deuterium oxide) on Viability of Mouse Sarcoma and Melanoma.

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Since the discovery of the isotope of hydrogen[1] much interest has developed concerning biological properties of heavy water. Lewis[2] found that heavy water (99% H^2_2O) completely inhibited the germination of tobacco seeds. Taylor, Swingle, Eyring, and Frost[3] showed that tadpoles of the green frog, *Rana clamitans,* died within one hour after being placed in the heavy water (92% H^2_2O). Macht and Davis[4] obtained a slight inhibiting effect on germination of *Lupinus* seeds with less concentrated heavy water (0.05% H^2_2O). On the other hand, Barnes[5, 6] has noted a stimulating effect of heavy water (0.05% H^2_2O) on *Spirogyra.*

It is known that neoplasms are generally more sensitive to deleterious agents than are normal tissues. Since normal tissues are killed by water containing deuterium in high concentrations, and biological effects are alleged for concentrations of heavy water as low as 0.05%, the question at once arises of what will be the effect of heavy water upon tumor tissues. This can be determined readily by exposing tumor fragments to the heavy water, and subsequently transplanting into host animals.

Two concentrations of heavy water were used—14.8 and 40%. These were samples used by La Mer, Eichelberger, and Urey[7] in their freezing point determinations. Before each experiment, the water was purified by repeated distillation from potassium permanganate. Since it was distilled very slowly, it was probably saturated with air from the condenser.

The tumors used were the mouse sarcoma 180, and the Passey mouse melanoma. Aseptic precautions were taken in all experiments.

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[1] Urey, H. C., Brickwedde, F. G., and Murphy, G. M., *Phys. Rev.*, 1932, **39**, 164, 864.

[2] Lewis, G. N., *J. Am. Chem. Soc.*, 1933, **55**, 3503.

[3] Taylor, H. S., Swingle, W. W., Eyring, H., and Frost, A. A., *J. Chem. Phys.*, 1933, **1**, 751; *J. Cellular and Comp. Physiol.*, 1933, **4**, 1.

[4] Macht, D. I., and Davis, M. E., *J. Am. Chem. Soc.*, 1934, **56**, 246.

[5] Barnes, T. C., *J. Am. Chem. Soc.*, 1933, **55**, 4332.

[6] Barnes, T. C., and Larson, E. J., *J. Am. Chem. Soc.*, 1933, **55**, 5059.

[7] La Mer, V. K., Eichelberger, W. C., and Urey, H. C., *J. Am. Chem. Soc.*, 1934, **56**, 248.

Into each of 2 weighing bottles were placed 2.5 cc. of Locke-Ringer solution* which was evaporated to dryness over a covered water bath. One residue was then dissolved in 2.5 cc. of ordinary distilled water and the other in 2.5 cc. of heavy water, thus making isotonic solutions. The solutions were buffered to pH 7.0,† approximately (checked colorimetrically), by adding 0.088 cc. of 0.2 M KH_2PO_4 and 0.052 cc. of 0.2 M KOH. Small pieces of tumor tissues (each weighing about 6 mg.) were placed in these solutions and left for 12 to 24 hours at 4-5°C. At the end of these periods of time, the tumor fragments were inoculated into mice. Parallel experiments were run with tumor fragments in ordinary distilled water and 40% heavy water, neither of which had been brought to isotonicity by the addition of the Locke-Ringer salts. This group of 4 sets involved 110 tumor implants.

The results showed that 40% heavy water has no effect upon the transplantability of the tumor fragments if isotonicity be maintained by a balanced salt solution. The tumor fragments which had been immersed in Locke-Ringer solution made up in ordinary and in 40% heavy water grew normally when transplanted into animals. Histological examinations of these treated tumor tissues showed no definite changes. On the other hand, the growth capacity of the mouse sarcoma 180 was markedly destroyed when subjected to hypotonic media for 24 hours, both in ordinary and heavy water (60 to 70% inhibition).

Experiments duplicating those with mouse sarcoma 180 were made with the Passey mouse melanoma. This group involved 108 tumor implants. The results obtained from these experiments were essentially the same as in the case of mouse sarcoma 180. The growth capacity of the melanoma was completely destroyed by 24 hours immersion in ordinary or 14.8% heavy water at pH 7.0. On the other hand, immersion in 40% heavy water or ordinary water solution, which were isotonic from the Locke-Ringer salts, did not affect the growth capacity of the mouse melanoma.

Barnes[5] has suggested " a stabilizing action of water containing the heavy isotope . . . . ", which he considers protective. Since exposure of the tumor fragments to hypotonic media is destructive, it is interesting to note if heavy water is less destructive than ordinary water.

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* A solution containing 0.15 moles of NaCl, 0.003 moles of KCl, and 0.003 moles of $CaCl_2$ per liter.

† The growth capacity of mouse sarcoma 180 was completely destroyed by immersion in a Locke-Ringer solution at pH 4.0 or 10.0. At pH 5.0, 69% inhibition and at pH 9.0, 61% inhibition occurred. Tumor fragments immersed in solutions at pH 6.0, 7.0 and 8.0 for 24 hours at 4-5°C. grew normally when implanted into mice.

Equal numbers of fragments of mouse sarcoma 180 were placed in 2.5 cc. portions of 14.8% heavy water and ordinary distilled water. These media had been buffered to pH 7.0 by means of 0.088 cc. of 0.2 M KH_2PO_4 and 0.052 cc. of 0.2 M KOH. The weighing bottles containing the tumor fragments (from 10 to 20 pieces in each bottle) were allowed to remain for definite intervals of time of 6 to 48 hours in the refrigerator at 4-5°C. At the end of different periods of time, the fragments were inoculated into mice. The results show that immersion in heavy water or ordinary distilled water for 6 hours is without effect; the tumors grew normally. After immersion for 12 hours, the rate of growth was slightly retarded and partial inhibition of takes (20% in both ordinary distilled water and heavy water). Similar treatment for 24 hours produced from 50 to 71% inhibition in both ordinary and heavy water. Exposures for 30 hours produced 80% inhibition and marked retardation of growths, and 48 hours resulted in complete destruction of growth capacity.

Gross and histological examination of a number of tumor tissues after immersion in the heavy water and ordinary distilled water showed essentially the same things: *i. e.*, the swelling of the tumor tissues, their jelly-like appearance, and the hydropic degeneration of the cytoplasm, with nuclear degeneration.

In a separate experiment, it was found that 24 pieces of tumor tissue before immersion in 14.8% heavy water (pH 7.5) weighed 0.1698 gm., and after immersion for 24 hours at 4-5°C. they weighed 0.2646 gm. (156%). In a simultaneous experiment, 24 fragments before immersion in ordinary distilled water (pH 5.3) weighed 0.1698 gm., and after immersion they weighed 0.2200 gm. (130%). The swelling was greater in heavy water, but the disparity may be due to the difference in pH.

Summary. The proliferating capacity of the mouse sarcoma 180 and the mouse melanoma was unaffected by heavy water (14.8 and 40% $H^2{}_2O$) when it contained salts of a Locke-Ringer solution in an isotonic amount.

The authors wish to express their indebtedness to Dr. Urey of Columbia University for the use of his laboratory in the manufacture of heavy water, and for his interest in the present study. We wish also to thank Dr. Failla whose interest made this work possible.

7266

Estimation of Anterior Pituitary-like Hormone in Cord Blood.

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In order to study the presence or absence of the anterior pituitary-like hormone in the fetal and placental circulations, and perhaps its effect on the fetal ovary determinations of the hormone were made upon the cord blood.

The blood specimens were obtained from the umbilical cords of 2 women immediately after the birth of the babies by severing the cords and allowing the blood from each of the cut ends to flow into separate vessels. The specimens obtained from the fetal side of the cut cords were considered to be fetal blood, and that from the maternal side, placental blood, or blood going from the mother to the fetus. The bloods thus obtained were allowed to clot and the serum only was used for the hormonal determinations. As relatively small quantities of serum could be obtained by this method, especially from the fetal side of the cord, the total quantities of serum obtained were injected. The immature rat was used as the test object, and a total of 4 rats were injected with the serum. In the first case of parturition 5 cc. of serum obtained from the placental end of the cut cord, and 5 cc. of serum obtained from the fetal end were injected into 2 immature rats respectively. In the second case 3 cc. of serum obtained from each end were injected on the same day.

The ovaries of the rats which received the placental blood serum in both the 5 cc. and 3 cc. quantities showed luteinized follicles (A.P.R. III). On the other hand, the ovaries of the rats which received the same quantities of serum from the fetal side of the cord (5 cc. and 3 cc. respectively) showed a negative response. Since 3 cc. of placental blood serum showed at least 1 R.U. of the anterior pituitary-like hormone, it is estimated that there are at least 165 R.U. per liter of blood entering the fetus during pregnancy. Aschheim and Zondek found 150 M.U. per liter. It is emphasized that only the "luteinizing" anterior pituitary-like factor was looked for. The Anterior Pituitary Reaction I was not considered positive. It is conceivable that if greater quantities of serum were used that a positive reaction may be demonstrable in the fetal blood. However, in the quantity here used (5 cc. of serum) a negative reaction was obtained.

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* **Herbert L. Celler Foundation Fellow.**

It is evident from the reactions obtained that the anterior pituitary-like hormone enters the fetal circulation through the cord but does not return in same concentration. In corroboration, Wislocki and Snyder[1] have demonstrated that the hormone fails to pass from the fetal circulation to the mother. It seems likely that it is fixed or destroyed in the fetal tissues.

To determine whether or not the hormone is stored in the liver, 108 gm. of liver obtained from a fetus dying *in utero* was extracted by means of 60% acid alcohol and the extract injected into[2] immature rats. The equivalent of 54 gm. (½ the total quantity obtained) was negative on 2 occasions. Apparently the hormone is not stored in the liver.

Examination of fetal ovaries fails to show any evidence of follicle maturation or luteinization. The uterus also shows no variation from the quiescent state. The hormone although entering the fetal circulation evidently does not act upon these structures. Further investigation is necessary to determine its fate in the fetus.

7267 P

Specificity of Enhancing Materials from Mammalian Tumors.

ALBERT E. CASEY.

From the Laboratories of The Rockefeller Institute for Medical Research.

That growth promoting substances are present in transplanted tumors has been known for a long time. But that they can exercise a profound influence over the metastatic phase of malignancy, that their effects in sensitizing the host to a malignant growth can be more or less permanent, or that they have species and tissue limitations in their activity is only now being demonstrated.[3] Thus, a filterable material from the Brown-Pearce rabbit tumor has been found to enhance every observed phase of the growth of this tumor, both local and metastatic. A species limitation has been noted in that the material from the rabbit tumor would not enhance the growth of mouse carcinoma 63 or mouse sarcoma 180. Rabbits treated with the material were still profoundly hypersusceptible to both local and metastatic phases of the Brown-Pearce tumor 7 months after the last injection.[2] The growth promoting material described by Haaland[3] and Leitch[4] in mouse carcinoma 63 has been found to enhance growths of the same tumor but has had no enhancing effect on the growth of the rabbit tumor or the growth of mouse sarcoma 180.

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[1] Wislocki and Snyder, PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 196.

[2] Zondek, B., *Hormone d. Ovariums u. des. Hypophysenvorderlappens.* Julius Springer, 1931, 200.

[3] Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 869; 1933, **30**, 674, 1025.

That enhancing materials are to be found in sarcomata seemed evident from the work of Flexner and Jobling[5] and of Chambers and Scott.[6] To test the specificity and biology of sarcoma enhancing materials, experiments were undertaken of which the first 3 are here reported. In each experiment tumor tissue from mouse sarcoma 180 was anaerobically refrigerated for 14-60 days at 24°F., minced and ground without sand, and emulsified with normal saline (dilution 1-10). In one experiment the emulsion was filtered through a Berkefeld "V" candle. Of this emulsion 0.1 cc. was injected into the left groin of 10 mice; 2 weeks later the 10 mice and 10 control mice not previously treated were inoculated in the same groin with fresh sarcoma 180. The incidence of primary tumors in 30 treated mice was 24 (80%) as compared with 17 (56.7%) among the 30 controls ($X^2 = 3.78$, $n = 1$, $P = 0.055$, probably significant); at 20 days the average cubic volume of the 24 tumors in the treated animals was 1.421 cc. as compared with 0.432 cc. for the 17 tumors among control animals (difference = 0.989 cc. ± 0.266, $t = 3.726$, $P = 0.01-$, a very significant result). In parallel experiments 30 additional mice were injected with the same sarcoma 180 enhancing material; 2 weeks later these mice and 28 additional control mice were inoculated with fresh mouse sarcoma 37. At 17 days 22 (75.8%) of the 29 treated mice had sarcomata as compared with 23 (82.1%) among the 28 control mice ($X^2 = 0.508$, $n = 1$, $P = 0.5$ not significant); the average of 22 tumors in treated animals was 0.716 cc. as compared with 0.9 cc. for 22 tumors among the controls (difference = 0.184 ± 0.236 cc., not significant). In the third experiment the sarcoma 180 enhancing material was tested for its activity on the growth of mouse carcinoma 63 in a different series of mice, and although one experiment is not conclusive, a slightly inhibiting instead of an enhancing effect was obtained. In mouse sarcoma 180 tissue there is, therefore, a material separable from the cells which has a profound enhancing effect on the incidence and growth of the same tumor but has no enhancing effect on the incidence and growth of mouse sarcoma 37 or of mouse sarcoma 63.

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[2] Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 666.

[3] Haaland, M., *Proc. Roy. Soc. of London*, 1910, **82**, 293; *Lancet*, 1910, **1**, 787.

[4] Leitch, A., *Lancet*, 1910, **1**, 991.

[5] Flexner, S., and Jobling, J. W., PROC. SOC. EXP. BIOL. AND MED., 1907, **4**, 156; **5**, 16. Jobling, J. W., *Monographs of The Rockefeller Institute for Medical Research*, 1910, **1**, 52.

[6] Chambers, H., and Scott, T. M., *J. Exp. Path.*, 1924, **5**, 1; 1926, **7**, 33; *J. Path. and Bact.*, 1932, **35**, 283.

In another series of experiments, the effect of the mouse carcinoma 63 enhancing material has been tried on mouse carcinoma 48.* Of 50 mice in 5 experiments treated with 0.1 cc. of the carcinoma 63 material 2 weeks before the inoculation of carcinoma 48, the incidence of primary tumors was 5 (10%) as compared with 10 tumors (24%) among the 49 control mice not treated ($X^2 = 0.095$, $n = 1$, $P = 0.8$, not significant); the cubic volume of the 4 tumors at 28 days in treated animals averaged 0.512 cc. as compared with 0.773 cc. for the 10 tumors in control mice (difference = 0.216 cc., not significant). In no single experiment was there any evidence of enhancement. On the contrary, there was a faint suggestion of an inhibiting influence in each of the 5 experiments. However, the low percentage of takes among the controls indicates that additional experiments should be performed.

By biological experimentation 3 distinct homologous tumor enhancing materials have been differentiated the one from the other, namely, that from the rabbit tumor, that from mouse carcinoma 63, and that from sarcoma 180. Each enhances growths of its own tumor but does not enhance growths of the other 2 tumors. Furthermore, since the mouse carcinoma 63 material did not enhance mouse carcinoma 48, and the sarcoma 180 material did not enhance mouse sarcoma 37, one might expect to find at least 2 biologically distinct carcinoma enhancing materials and 2 biologically distinct sarcoma enhancing materials in the same animal species. The only other explanation would be that sarcoma 37 is really a carcinoma and carcinoma 48 a sarcoma. The work is still in progress.

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* The author is indebted to Dr. Wm. H. Woglom of the Crocker Institute for this tumor.

7268 P

A Persistent Hypersusceptibility Induced in Rabbits with an Homologous Tumor Material.

ALBERT E. CASEY.

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A single injection of a filterable material from homologous tumor tissue prepared by prolonged anaerobic refrigeration will render the rabbit host more susceptible to the inoculation and increase the malignancy of the Brown-Pearce rabbit tumor. The dosage employed may vary from 0.2 to 0.5 cc. of a 1-5 dilution of the material.[1] The material is equally active when inoculated either 2 weeks before or 2 weeks after the tumor inoculation. In a previous experiment injection of the material 3 times at 2 month intervals led to increased susceptibility instead of to an immunity to this tumor.

This paper reports a second series in which 5 male rabbits were injected 8-10 times at intervals varying from 2 weeks to 2 months. The rabbits were then permitted to rest for 7 months, during which period no treatment was given. At the end of this period the 5 rabbits (experimental group) and 14 control rabbits (adults of the same sex, size, origin, and breed but almost a year younger) were inoculated intratesticularly with fresh Brown-Pearce tumor. The experiment was terminated at 2 months. The incidence of primary testicular tumors was 4 (80%) for the experimental group as compared with 11 (78.6%) for the control group; the cubic volume of the 4 primary tumors at autopsy in the experimental group was 15.5 cc. as compared with 10.0 cc. for the 11 primary tumors in the control group; the incidence of metastatic tumor in the experimental group was 4 (80%) as compared with 9 (64.3%) for the controls; the cubic volume of the metastatic tumor per animal with metastases was 175 cc. in the experimental as compared with 38.3 cc. for the control group; the average number of metastatic foci was 22.0 for the experimental as compared with 9.0 for the control group; the total mortality for all animals inoculated was 73.4% in the experimental as compared with 46.4% for the control group; the total mortality for all animals with tumor was 91.8% in the experimental as compared with 59.1% for the control group; the average longevity for all 5 animals in the experimental group was 50.8 days as compared with 58.6 days for the 14 animals in the control group.

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[1] Casey, A. E., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 869; 1933. **30**, 674, 1025; 1934, **31**, 663.

The age discrepancy of almost 12 months between the control and experimental groups will not explain the increased susceptibility obtained, for previous experience with this tumor indicates that older animals develop tumor more easily but actually have fewer metastatic foci and a smaller amount of metastatic tumor than younger adult animals within a given period of time. Also the older adults used were not old in an absolute sense but vigorous young adults in good physical condition. In more than 75 groups of normal adult male rabbits (including more than 350 animals) inoculated with this tumor during the past 5 years, none has had the number of metastatic foci or the volume of metastatic tumor as was found in the experimental group of the present series. One animal of the 5 had the largest volume of metastatic tumor, and another as large a number of metastatic foci as has been recorded in this laboratory. This includes not only the 350 normal animals of various breeds and hybrids inoculated, but also more than 150 animals treated with the homologous tumor material either 2 weeks before or 2 weeks after the tumor inoculation. Since 2 animals among 5 had 2 quantitative criteria of malignant disease such as occur only once in more than 500 times, and the group as a whole a value such as occurred among normal control groups but once in 75 times, the evidence of increased susceptibility to malignant disease seems conclusive. The effects of the homologous tumor material were, therefore, profoundly evident after a period of 7 months. The experiment deserves repeating to determine whether such a lasting hypersusceptibility can regularly be induced. If so, prolonged treatment with the material in a cell free state, either alone or combined with an irritant might eventually give rise to a tumor of the same kind.

7269 C

Is Guinea Pig Follicular Fluid Auto-Antigenic?*

MARGARET G. SMITH. (Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine, St. Louis.

In reporting some anaphylactic studies with mammalian follicular fluid Lyons and Van de Carr[1] claimed (1) that the follicular fluids of 4 mammals studied (sow, cow, mare and ewe) were closely related immunologically; (2) that guinea pig follicular fluid was auto-antigenic and that fatal anaphylaxis could be produced at a definite time in the oestrous cycle (tenth-sixteenth days) in normal guinea pigs on primary injection of heterologous follicular fluid; (3) that guinea pigs artificially sensitized to heterologous follicular fluid proteins are temporarily desensitized during the early part of the oestrous cycle by the liberation of their own follicular fluid.

While the first of these claims seemed not improbable from what is known about certain non species-specific or relatively species specific antigens, the others seemed so remarkable and, if substantiated, of such biological importance that we were led to try to verify them.

A number of healthy female guinea pigs weighing 300-350 gm. were observed to determine the length of the oestrous cycles. Only those animals having a fairly regular cycle, 15 to 17 days were used. The test injections were made into a large leg vein rather than intracardially, to avoid the danger of a pericardial hemorrhage. The beef and hog follicular fluid used in these experiments was collected as soon as possible after the animals were slaughtered. It was centrifuged and either used immediately or kept frozen until needed, at which time it was thawed slowly at room temperature.

Twenty female guinea pigs were injected intravenously (Table I) with varying amounts of one of 3 different lots of beef follicular fluid. Fifteen of these animals were injected at periods later than the tenth day after the beginning of the estrous cycle, 5 at earlier periods. Eight additional female guinea pigs were similarly injected with one of 2 lots of hog follicular fluid, 5 of these on days later

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* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Lyons, W. R., and Van de Carr, F. R., *Arch. Path.*, 1930, **9**, 1.

than the tenth of the estrous cycle, and 3 earlier in the cycle. The results of the injections with hog follicular fluid are recorded in Table II.

TABLE I.
Reactions of Normal Guinea Pig to Beef Follicular Fluid.

Amt. of fluid used cc.	Lot	Day of cycle	No. animals (20)	Results
¼	A	15	1	0
½	A	14	1	0
½	A	15	2	0
¾	A	13	1	0
1	A	15	1	0
1	B	12	1	[illegible]
1	B	14	2	0
2	B	3	1	Died in about 1 hr.
2	B	14	2	0
2	B	15	1	Lay quietly on side; recovered
3	B	6	1	Lay on side; recovered
3	B	9	1	Died
3	B	14	1	[illegible]
3	C	6	1	0
3	C	15	3	0

TABLE II.
Reactions of Normal Female Guinea Pig to Hog Follicular Fluid.

Amt. of fluid used cc.	Lot	Day of cycle	No. animals (8)	Results
3	A	3	1	0
3	A	9	1	0
3	A	13	1	0
3	A	14	1	0
3	B	4	1	0
3	B	12	1	0
3	B	15	2	0

With the beef follicular fluid no difference was seen in the reactions of animals in the early and those in the late stages of the estrous cycle, although Lyons and Van de Carr produced fatal anaphylaxis with as little as ½ cc. of hog follicular fluid during the later part of the cycle. With one lot of the beef follicular fluid, lot B, amounts of 2 cc. and 3 cc. seemed toxic to both groups of animals. However, none of the reactions were characteristic of typical anaphylaxis. Trembling, roughening of the hair, and collapse, in some animals, and death in 3 cases, were seen but not the sneezing, gasping and convulsive movements of anaphylaxis. With lot A and C no toxic effects were noted on intravenous injection.

Likewise, with the hog follicular fluid no difference was seen between the reactions of the animals injected early and those injected late in the estrous cycle. With neither of the 2 lots of hog follicular fluid were any toxic reactions seen with doses as great as 3 cc.

To test the possibility of cross immunization between follicular fluids from different species, each of 13 young male guinea pigs weighing approximately 350 gm. was given a sensitizing dose of 2 cc. of hog follicular fluid intraperitoneally, and each of 18 young male guinea pigs the same amount of beef follicular fluid intraperitoneally. Twenty days later 6 animals in each group were tested with the homologous fluid. The remaining 19 sensitized animals were tested with the heterologous fluid. All those animals which received homologous fluid in the test injection showed very definite and characteristic anaphylactic reactions, even with amounts of fluid as small as 1/8 cc.

Animals sensitized to hog follicular fluid, receiving beef follicular fluid showed characteristic anaphylactic reactions only if the amounts injected were as great as 1½ cc. Two non-sensitized male guinea pigs used as control showed no reaction to the intravenous injection of 2 cc. of the same beef follicular fluid.

In the group of animals sensitized to beef follicular fluid and receiving test doses of hog follicular fluid, 4 tested with 3 cc. amounts all showed marked anaphylactic reactions. Three of 6 animals receiving 2 cc. amounts showed marked reactions; the 3 others showed little or no reactions. The 2 animals receiving 1 cc. showed no reaction. Of 5 non-sensitized male controls one, receiving 3 cc. of hog follicular fluid, died; the others receiving as much as 2 or 3 cc. showed no change.

Thus there was demonstrated a definite cross anaphylaxis between cattle and hog follicular fluids, although rather large amounts of fluid (1½ cc. or more) were necessary for this purpose. On the other hand anaphylactic reactions were obtained with as little as 1/8 cc. of the homologous fluid.

Four female animals sensitized to hog follicular fluid all gave, after 20 days, characteristic anaphylactic reactions resulting in death when 2.5 cc. of hog follicular fluid was injected intravenously, although 3 of these animals received the test injection on the second day of the oestrous cycle, a time at which Lyon and Van de Carr claim that the female guinea pig is desensitized by the liberation of its own follicular fluid.

While we have thus been able to demonstrate a certain degree of relationship, as manifested by means of the anaphylactic reaction, between the follicular fluids of cattle and hogs, there was no indication of an auto-antigenic character of guinea pig follicular fluid, as claimed by Lyons and Van de Carr.

7270 P

An Attempt to Determine the Blood Groups of Mummies.

WILLIAM C. BOYD AND LYLE G. BOYD. (Introduced by S. B. Hooker.)
From the Evans Memorial (Massachusetts Memorial Hospitals), Boston.

The authors have shown previously[1] that dried human muscle may be used to determine the blood group of the person from which it came. The extension of the study to mummified tissue suggested itself, as information which might be thus obtained would be of interest in connection with the problem of the origin of the human blood groups and might be of value to anthropologists and archeologists. The present paper is a preliminary report of this work.

The technic was as described in the above reference. Anti-A and anti-B testing fluids, prepared by specific adsorption of immune rabbit sera, and also human sera of types B and A were used. These were adjusted in strength so that they agglutinated "+" at a dilution of 1:4 and "±" at 1:8. About 0.3 ml. was placed on each sample of about 0.05 gm. of finely ground mummified tissue. After thorough mixing and standing overnight in the icebox, the supernatants were allowed to come to room temperature and tested against A and B erythrocytes. The results are given in Table I.

It is evident that, in contrast to the American material, some of

TABLE I.
Tests for Adsorption of Agglutinins by Mummified Tissue.

I. American Indian Specimens

Test Fluid	Test Cells	Specimen No. 1	2	3	4	5	6	7	8	9
Anti-A	A cells	+	+	+	+	+	+	—	+	+
Anti-B	B "	+	+	+	+	+	+	+	+	+
Type B serum	A "	+	+	+	+	+	+	—	+	+
" A "	B "	+	+	+	+	+	+	+	+	+

II. Egyptian Specimens

Test Fluid	Test Cells	Specimen No. 1	2	3	4	5
Anti-A	A cells	—	—	—	—	—
Anti-B	B "	—	—	—	—	—
Type B serum	A cells	+	+	+	+	+
" A "	B "	—	+	—	—	+

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[1] Boyd, W. C., and Boyd, L. G., *Science*, 1933, **78**, 578; *J. Immunol.*, in press.

the Egyptian material, in spite of its great age, seems to remove both of the kinds of anti-B agglutinin used, and thus displays a specific adsorptive power. It seems quite possible that the Landsteiner blood groups can be determined by such methods; it is unfortunate that M and N do not seem to be demonstrable in muscle.

Work is now in progress to establish more definitely the presence of the agglutinogen B in these specimens, and to determine if an anti-O serum[2] can be prepared which will make possible the decision between a type O and a specimen which has simply lost all of its agglutinogens because of deterioration. Also, it would obviously be desirable to test a great many more samples, and the authors hope to do this if sufficient material can be obtained.

Thanks are due to the National Museum, The American Museum of Natural History, The Boston Museum of Fine Arts, Dr. J. Bronfenbrenner, Prof. C. Kluckhohn, and Dr. L. C. Wyman for material, and for assistance in obtaining material to The National Research Council and to Mr. D. Scott and Dr. E. A. Hooton, of the Peabody Museum.

7271 P

Further Studies on the Coagulo-Flocculation Test for Malignant Tumors.

EMIL WEISS.

From the Department of Bacteriology and Preventive Medicine, University of Illinois, College of Medicine, Chicago.

A modified procedure of the coagulo-flocculation test for malignant tumors[1] is described, based on additional studies of protein fractions of normal serums, those in various diseases and malignant tumors. The essential constituents are: (1) Diluted blood serum inactivated at 60°C. for ½ hour, which should be neither contaminated nor hemolized. (2) The antigen is prepared by extracting finely ground beef heart with 95% alcohol in the ratio 1:10 for 3 days at 37°C. and overnight at room temperature and then filtering. (3) A saturated watery solution of sodium chloride.

The serums are diluted with water before their use in the test according to the percentage of hemoglobin. Dare's hemoglobinometer is used as a standard, and to the percentage of hemoglobin obtained 10 is added and the sum is divided by 20, which gives the dilution for the respective serum. (For instance, the reading was 70% plus 10 = 80 ÷ 20 = 4. The dilution of the serum in this case would be 1:4.) Where the Dare reading is 45% or less, the serums should be diluted only to 1:3. Tallqvist's scales can be used if their average reading is previously compared with Dare and the difference taken into consideration in the calculation of the dilutions of the serums. For instance if the Tallqvist scale shows a 10% higher reading than the Dare, nothing is added to the hemoglobin reading before dividing with 20.

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[2] See references in Sasaki, H., *Z. f. Immunitätsf.*, 1932, **77**, 101.

[1] Weiss, E., *Arch. Path.*, 1932, **13**, 106.

The proper dilution of the serum can be determined also from the red blood count by the following formula: $\frac{\text{red blood count}}{1{,}000{,}000} - .5$ (i. e., if the red blood count was 4,500,000, then 4,500,000/1,000,000 = 4.5 — .5 = 4.0.) The dilution of the serum in this instance would be 1:4. Dilutions below 1:3 are not made.

Titration of antigens is carried out as follows: Increasing amounts of undiluted antigen (.16 cc., .18 cc., .20 cc., .22 cc., .24 cc., etc.) are placed in the corresponding tubes of 2 rows (each with 8 tubes) of a rack. In each tube of the first row 0.6 cc. of the diluted malignant serum are added and each tube of the back row receives 0.6 cc. of the diluted normal serum. The tubes are thoroughly shaken and then placed in a water bath for 5 minutes at 60 C. After the incubation each tube is diluted with 1.5 cc. of saturated solution of sodium chloride and the results are recorded. The largest amount of antigen which causes only turbidity in the normal tube and a distinct flocculation in the malignant tube is selected as the proper amount for the test (= titre). The titrated amount of antigen should be also tested with syphilitic, jaundice and anemic serums. The titre remains the same for an indefinite period if the antigen is properly preserved.

The routine test: Wassermann tubes are placed in 2 rows in the racks. The tubes of the first row are used for the main test with the unknown serums and also for the malignant, syphilitic, jaundice and anemic controls. The last tube in the first row contains the antigen control. The tubes in the second row serve as the serum controls for the unknown serums and also for the malignant, syphilitic, icteric and anemic serums. The titrated amount of the diluted antigen is placed in each tube of the first row. The corresponding amount of water is placed in all tubes of the second row. Six-tenths cc. of each diluted serum are added to one tube in the first row and an equal amount of the same serum to the tube behind in the second row. Six-tenths cc. of water (instead of serum) are added to the antigen control. The ingredients of the serum controls should be the same as those used for the unknown serums. All tubes are then shaken and placed in a water bath at 60°C. for 5 minutes. After the incubation each tube is diluted with 1.5 cc. of saturated solution of sodium chloride and the results are read. If the required amount of the unknown serum is not available, the test may still be performed successfully if the remaining constituents for the reaction are decreased proportionately.

Controls: The following controls are necessary each time the test is carried out: (1) antigen control, (2) serum control (each serum should have a serum control), (3) malignant, syphilitic, icteric and anemic controls.

Interpretation of the results: The controls should be examined before making readings of the unknown serums. The malignant control should show a thick layer of coagulated serum floating on the surface of the salt solution, which contains many large floccula. All other controls should remain uniformly turbid. One tube is read for each unknown serum. Tubes with a distinct flocculation or showing in addition a layer of suspended coagulated serum on the surface of the saline are read as strong positive. Tubes with a fine flocculation are read as weak positive. Tests with doubtful flocculations are repeated. Uniformly turbid tubes are read as negative.

This test applies to all types of malignant tumors. The modification of the original procedure raised the sensitiveness of the test from 79% strong positive reactions to 85% and with the weak positive reactions to 89%.

7272 C

Utilization of Inulin from Arctium Lappa and Certain Soluble Inulins by the Rat.

C. JELLEFF CARR* AND JOHN C. KRANTZ, JR.

From the Department of Pharmacology, School of Medicine, University of Maryland.

Since 1874[1] inulin-containing plants have been suggested for use in the diabetic diet as substitute carbohydrates. Okey,[2] studying the influence of digestive juices on inulin, found it to be hydrolyzed partially by the hydrochloric acid of the stomach and subject to marked decomposition by the intestinal flora, and concluded that human feces contains an enzyme capable of hydrolyzing inulin into a reducing sugar.

The authors showed that the inulin from *Arctium lappa* (Burdock root) was utilized by demonstrating its protein-sparing action on the diet of dogs[3] and its capacity to store additional glycogen in the liver of the rat.[4] A physicochemical study of the inulin from *Arctium lappa* was also conducted in conjunction with this investigation.[5]

Yanovsky and Kingsbury[6] prepared inulin from 3 new sources, 2 plants of the Liliaceae and one of the Compositae family, and observed that the inulins obtained from these sources were soluble in water and dilute alcohol. They later submitted quantitative data on the solubility of these inulins.[7]

It occurred to the authors that it would be interesting to compare the absorption and utilization of the soluble inulins with the insoluble inulin obtained from *Arctium lappa*. The authors are indebted to Doctors Yanovsky and Kingsbury of the Carbohydrate Division, Bureau of Chemistry and Soils, United States Department of Agriculture, for a liberal supply of inulin from Camas roots *(Quamasia quamash)* and from the Wild Onion *(Allium nuttallii)*.

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* Emerson Fellow in Pharmacology.

[1] Külz, E., *Beitrage zur Pathologie und Therapie des Diabetes Mellitus*, Marburg, 1874, 130.

[2] Okey, R., *J. Biol. Chem.*, 1919, **34**, 149.

[3] Krantz, J. C., Jr., and Carr, C. J., *J. Pharm. and Exp. Therap.*, 1931, **43**, 187.

[4] Krantz, J. C., Jr., and Carr, C. J., *J. Pharm. and Exp. Therap.*, 1931, **41**, 83.

[5] Krantz, J. C., Jr., and Carr, C. J., *J. Phys. Chem.*, 1931, **35**, 756.

[6] Yanovsky, E., and Kingsbury, R. M., *J. Am. Chem. Soc.*, 1931, **53**, 1597.

[7] Yanovsky, E., and Kingsbury, R. M., *J. Am. Chem. Soc.*, 1933, **55**, 3658.

Each of these substances served as food in the diet of the North American Indian.

Male white rats from 75 to 150 gm. were fasted for a period of 48 hours and then placed in small individual cages. The control rats were fed cacao butter for a period of 72 hours. The experimental rats were fed mixtures of inulin and cacao butter containing one-third inulin. After this period the amount of food ingested was determined by difference. The rats were then killed by exsanguination. The liver-glycogen was determined immediately by Pflüger's[8] method. The tissue-glycogen was determined by the method used by Cori and Cori[9] modified by Carr *et al.*[10] The reducing sugars formed by hydrolysis, as well as the carbohydrate in the alimentary tract and feces, were determined by the Munson, Walker method.[11] The results are given in Tables I and II.

TABLE I.
Control Rats.

Group Nb.	No. of Rats	Wt. of Rats, gm.	Food Ingested		Reducing Sugar After Hydrolysis Tract, gm.	Reducing Sugar After Hydrolysis Feces, gm.	Wt. of Livers, gm.	Wt. of Tissues, gm.	Liver-Glycogen, %	Tissue-Glycogen, %	
			Type	Amount							
1	2	138- 73	Cacao Butter	12.5	.00	.00	6.90	161	.09	.20	
2	2	150-147		14.1	.00	.02	8.90	234	.36	.11	
3	2	102-101		12.4	.04	.05	8.20	165	.03	.09	
4	2	107- 97		10.5	.01	.01	6.80	171	.11	.10	
5	1	108		8.2	.00	.03	3.50	78	.24	.04	
6	2	115-148		17.5	.08	lost	8.50	192	.12	.15	
									.16	.12	Mean

In Table II no correction was made for the tissue-glycogen as these values obtained when the animals were fed the inulins were practically identical with the control animals.

An examination of Tables I and II indicates the capacity of all 3 types of inulin to be absorbed and utilized. The amount of glycogen present in the livers is unusually high for animals fed inulin. Bodey

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[8] Cole, S. W., Practical Physiological Chemistry, Baltimore, 7th Edition, 1926, 171.

[9] Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1926, **70**, 557.

[10] Carr, C. J., *et al.*, *J. Biol. Chem.*, 1933, **102**, 721.

[11] Munson, T. S., and Walker, P. H., *J. Am. Chem. Soc.*, 1906, **28**, 163.

TABLE II.
Experimental Rats.

			Food Ingested		gm.								
Group No.	No. of Rats	Wt. of Rats, gm.	Type	Amount	Inulin in Feces	Inulin in Tract	Inulin equivalent of liver Glycogen	Liver-Glycogen %	Tissue-Glycogen %	Inulin Utilized by Difference	Inulin Utilized %	Inulin Absorbed %	
1	2	120-122	Inulin* from	6.6	.43	.40	.29	3.11	.12	5.50	83.2	87.3	
2	2	125-125	*Arctium lappa*	6.9	.68	.46	.34	3.86	.16	5.45	78.6	83.3	
3	2	128-126	and Cacao Butter	6.0	.24	.17	.24	2.79	.14	5.35	89.2	93.2	
4	2	122-124		5.5	.24	.23	.29	2.42	.08	4.73	86.0	91.4	
5	2	120-122		4.3	.11	.09	.20	2.20	.13	3.93	90.8	95.4	
								2.87	0.13		85.7	90.1	Mean
1	2	144-118	Inulin from	10.8	.45	.95	.15	1.61	.12	9.25	85.5	87.1	
2	2	130-123	Allium and	8.3	.09	.80	.10	1.06	.08	7.31	88.2	89.2	
3	2	150-141	Cacao Butter	10.7	.55	.00	.07	0.68	.19	10.10	94.4	94.8	
4	2	140-147		10.4	1.34	.00	.11	1.04	.17	8.95	86.0	87.2	
								1.10	0.14		88.5	89.5	Mean
1	2	104-112	Inulin from	10.3	.55	.12	.18	1.77	.10	9.45	91.6	93.4	
2	2	124-104	Camas and	6.9	.23	.08	.23	2.76	.09	6.36	92.3	95.4	
3	2	113-127	Cacao Butter	10.4	.69	.22	.19	1.68	.09	9.30	89.3	91.2	
4	2	119-105		10.6	.43	.30	.23	2.40	.10	9.64	90.7	93.1	
5	2	113-120		8.7	.29	.05	.40	3.82	.07	7.96	91.5	96.2	
								2.48	0.09		91.2	93.7	Mean

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*The inulin used was extracted from *Arctium lappa* for the authors by Burroughs Bros. Co. of Baltimore, Md. The product was purified to meet the standards set forth by the authors in reference 5.

et al.[12] found the inulin from the Jerusalem artichoke produced additional glycogen-storage in the white rat. The average glycogen content found by these workers was 0.69%. It will be observed that the percentage of liver-glycogen in the experiments with rats fed the inulin from Allium are less than half the values obtained when the Camas and Burdock inulins were fed. Owing to the tremendous variations inherent to the experiment the authors do not interpret this as meaning that Allium is less capable of forming liver-glycogen than the other 2 inulins.

In all cases, however, the high percentage of absorption from the alimentary tract, accompanied with a high percentage of utilization is significant. With feeding experiments on dogs the authors found 86% absorption when Burdock inulin was fed.

Summary. In the white rat, the absorption and utilization of the insoluble inulin from Burdock and the soluble inulins from Camas and Allium, show no significant difference.

7273 P

Further Studies Concerning Testicular Function.

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Fifty-four albino rats were divided into 4 groups: (1) normal controls, (2) castrated animals, (3) castrated animals injected with androtin (male sex hormone prepared from urine) and (4) normal animals injected with androtin. The androtin was prepared by extracting male urine with fat solvents. The injected rats each received from 8 to 10 bird units of androtin daily.

The results are shown in Table I. The weights of these animals are expressed in grams and the weights of the formalin-fixed organs as percentages of the total body weight. In the glands other than the sex glands, the difference between the weights of the glands in the control animals and of those in the injected normal animals is not sufficiently great to be of any significance. The seminal vesicles, the ventral and dorsal prostates all show an increase in weight of approximately 100% after the injection of androtin into normal rats. Other workers have stated that hormone prepared from the testes causes testicular degeneration.[1] If there was any testicular degeneration in the animals injected with androtin, it was not discernible by gross examination. The weights of the epididymises of the injected rats are slightly increased above those of the normal.

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[12] Bodey, M. G., *et al.*, *J. Biol. Chem.*, 1927, **75**, 715.

TABLE I.

Effect of Injection of Hormone into Normal and Castrated Rats.

	Normal	Normal injected with male sex hormone for 20 days	Castrated	Castrated and injected with male sex hormone for 21 days	Castrated	Castrated and injected with male sex hormone for 21 days
Duration of Exp.		20	22 days	21 days	85 days	85 days
No. of animals	9	7	6	12	10	10
Original wt. of animals	211	223	207	204	195	174
Final ″ ″ ″		226	200	208	195	194
Testes	1.050	.986				
Epididymis	.319	.353				
Seminal vesicles	.231	.366	.043	.306	.032	.165
Ventral prostate	.142	.256	.015	.189	.017	.085
Dorsal ″	.044	.076	.010	.061	.010	.035
Thyroid	.014	.013	.013	.015	.014	.014
Adrenals	.012	.016	.013	.015	.025	.016
Pituitary	.003	.004	.004	.004	.006	.006
Thymus	.090	.080	.098	.072	.097	.086

In mature rats, the effects of castration have been prevented for 3 weeks by the injection of androtin. In that length of time, the secondary sex glands become almost completely atrophic in untreated, castrated animals. However, during the same period, there are no significant changes in the thyroid, adrenals, pituitary or thymus glands, with the possible exception of the pituitary, which may be slightly hypertrophic.

Because the atrophy of the secondary sex glands commences within 5 days after castration and progresses very little, if any, after 3 weeks, much work previously done on the castrated rats has been limited to a 3-week period. We have studied the organs of rats which have been castrated for 76 to 107 days, averaging 85 days, and have also studied a similar group which were castrated for 85 days and treated for the last 21 days with testicular hormone. Following castration, atrophy of the secondary sex glands occurred. Grossly, the atrophy is not much more marked than that which was observed in animals examined at the end of 3 weeks. At the end of 85 days, the weight of the thyroid was not changed. The adrenals and the pituitary showed very definite and unquestionable hypertrophy at the end of this longer period, both being increased in weight approximately 100%. On injecting these animals with androtin, the secondary sex glands commence to regenerate. No hypertrophy of the adrenals was observed in 4 of the animals which were injected with androtin for the last 21 days of an 85-day period, and in the other 6, the hypertrophy was so slight as to be questionable. In this group of castrated animals which had an adequate amount of hormone to maintain the prostate and to reduce the hypertrophy of the adrenals, the pituitary hypertrophy was not altered during a period of 3 weeks.

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[1] Moore, C. R., *Proc. Second Int. Cong. Sex Res.* (London), 1931, 293.

7274 C

Spread of Poliomyelitis Virus from the Gastrointestinal Tract.*

JOHN A. TOOMEY.

From the Department of Pediatrics, Western Reserve University, and the Division of Contagious Diseases, City Hospital, Cleveland, Ohio.

Flexner and Lewis[1] first showed that poliomyelitis could be transmitted by intranasal instillation, the spread of the virus being along the olfactory nerve to the brain. The olfactory nerve, however, is not the only unmedullated one situated in the nasal area, for it is precisely here under the mucosa that the 1500 cells of nervus terminalis are placed,[2] composing a network of cells and fibers as might be found in the myenteric or submucous intestinal sympathetic.[2, 3] The position of the plexus of the nervus terminal near the embryological juncture of the stomodeum and the possible upper tip of the mesenteron, *i. e.,* the upper end of the foregut of the endodermal tube is significant, since the other portion of the alimentary tract, the hind gut, also possesses the same kind of unmedullated nerve fiber plexuses. If the virus has a facultative or almost an obligate affinity for grey fibers, this area should also provide an easy portal of entry. Perhaps failure to produce the disease after the virus of poliomyelitis had been introduced into the gastrointestinal tract was because the virus never approximated the grey fibers.

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* Expenses defrayed in part by a grant from the Marion R. Spellman Fund, The Cleveland Foundation.

[1] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.,* 1910, **54**, 1140.

[2] Brookover, C., *J. Comp. Neur.,* 1917, **28**, 2.

[3] Huber, C. G., and Guild, S. R., *Anat. Rec.,* 1913, **7**, 253.

In other experiments where the abdomen of a monkey or a rabbit had been opened and the intestine watched after the introduction of the virus through a tube or needle, it was observed that the material was usually swept out of the small intestine into the colon. This was followed by a period of spastic hyperactivity in the small intestines. No approximation could have occurred. The following technic was then devised. The abdominal cavity of a completely anesthetized *Macacus rhesus* monkey was exposed through a midline incision. The small intestine was clamped by a pair of intestinal clamps a few inches above the ileocecal valve. Another pair clamped the intestines about ½ to over one foot above the lower instrument; 50 to 100 cc. of a 1% emulsion of potent poliomyelitis virus were introduced into the isolated portion of the intestinal canal through a 24 gauge needle until the gut was ballooned out. It was very important to introduce enough virus so that the intestines would be tensely dilated. The clamps were kept on until the pinch reflex had disappeared. The intestines were returned to the abdominal cavity and the incision sewed tightly and bandaged. Two animals who were tested in this manner developed typical poliomyelitis.

One might contend that the animal might have regurgitated some virus and thus have infected the olfactory area even though the injection had been made into the small intestine. Since the postganglionic fibers of the thoracolumbar sympathetic system are unmyelinated, at least to the large abdominal plexuses, and those of the intestinal blood vessel unmyelinated as far as the vertebral ganglia themselves, there was no reason why the disease should not have been produced by a subserosal injection of the virus. Such a procedure would absolutely rule out the possible contamination of the olfactory area. Accordingly, the abdomen was opened in the manner previously described and 10 to 20 cc. of a 1% suspension of potent poliomyelitis virus was injected subserosally at multiple points. The needle could be seen distinctly as it was inserted beneath the outer intestinal coat, and when the injection was started, a whitish, crescentic, dimpled lesion resulted which crackled as it extended. After a few minutes, the lesion became deep red or wine colored. What little leaking occurred, went outside and not into the gastrointestinal tract. The abdomen was then sewed tightly and bandaged. Two monkeys were injected in this manner and both developed poliomyelitis.

7275 C

Histological Changes in the Hypophysis Produced by Chronic Administration of Hypophyseal Extracts.

J. B. COLLIP, H. SELYE AND D. L. THOMSON.

From the Department of Biochemistry, McGill University, Montreal.

Twenty-two female rats were injected daily from their twenty-first day of life with various hypophyseal extracts. These preparations were separated from the filtrate obtained by the calcium-phosphate adsorption process, as described in a previous communication.[1] This filtrate was treated with saturated ammonium sulfate and the resulting precipitate was further purified by precipitation with 50% acetone or, in some instances, 70% alcohol. These extracts were rich in thyreotropic hormone but they also contained some gonadotropic hormone. After 6 to 10 weeks' treatment with these preparations (50 to 100 units thyreotropic daily), the animals were killed and their organs examined histologically

It was found that the ovaries showed definite signs of involution. Mature follicles or fresh corpora lutea were never present, and the ovary consisted mainly of interstitial tissue.

The anterior lobe of the hypophysis showed marked degenerative

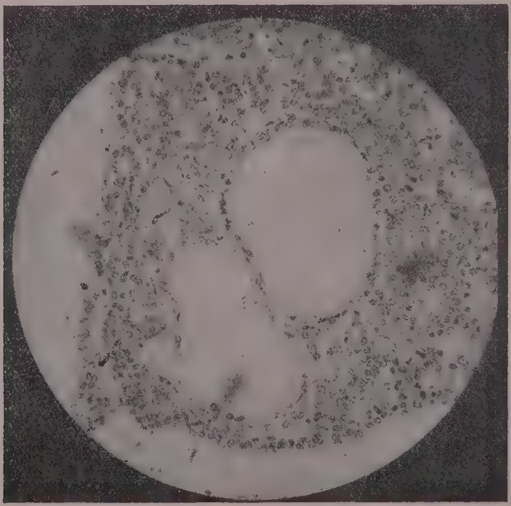

FIG. 1. Pituitary showing cysts.

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[1] Collip, Selye and Thomson, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 544.

changes. Cells of unusual size, having an excentric nucleus and a large vacuole in the cytoplasm, were observed. These elements are very similar to the so-called "castration cells" or the cells observed after thyroidectomy. In many cases hemorrhages were seen in the anterior lobe, and large cysts lined with epithelium and filled with colloid were frequently observed.

These findings show that marked histological changes of a degenerative type may be produced in the hypophysis by the chronic administration of hypophyseal extracts.

7276 C

Production of Pseudo-Pregnancy by Mechanical Stimulation of the Nipples.

H. SELYE AND T. MCKEOWN. (Introduced by J. B. Collip.)

From the Department of Biochemistry, McGill University, Montreal.

We have previously described experiments on lactating rats in which we found that the nervous stimulus of suckling could maintain the dioestrus of lactation after all galactophores had been transcised.[1, 2] It was also observed that in such animals, from which escape of milk to the litter was prevented, the mammary gland did not undergo rapid involution, but remained in an actively secreting state for some time in spite of the accumulation of the products of secretion.

The escape of milk is therefore not an essential condition for the continuation of dioestrus or for the maintenance of secretory activity in the mammary glands. Accordingly, the questions arise whether the influence of suckling could produce dioestrus in the absence of actively lactating glands and whether, since suckling will maintain the secreting gland, it could lead to a regeneration once involution has set in.

In order to answer these questions we performed the following experiments on rats.

The animals used fall into 3 groups: first, adult virgin rats; second, animals whose litters have been weaned at the end of a normal lactation and which have been seen to be normally cyclic for at least 3 cycles; and third, lactating mothers deprived of their litters

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[1] Selye, H., *Am. J. Physiol.*, 1934, **107**, 535.

[2] Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinology*, 1934, in press.

on the third day postpartum and studied daily until one or 2 oestrus cycles had appeared. To all animals a strong suckling stimulus was applied, and in order to keep the young alive they were exchanged with the well nourished litter of a normally lactating mother on alternate days, the young of the normal mother being transferred on these days to the experimental animal. Thus the rats under observation were continuously exposed to the suckling stimulus.

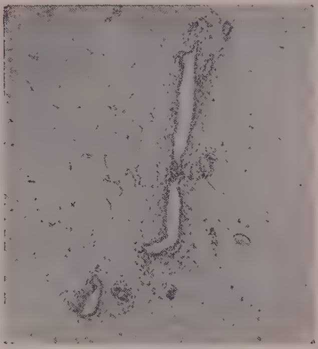

FIG. 1.
Mammary gland of an adult female rat. Only ducts are present.

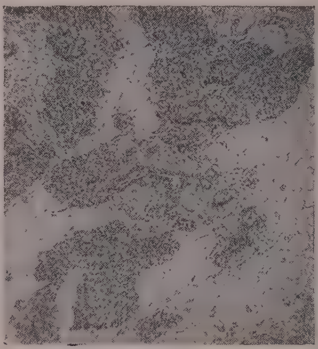

FIG. 2.
Mammary gland of the same rat in early stage of pseudo-pregnancy induced by suckling Alveoli form around the ducts.

There were obvious technical difficulties. On the theory of cer-

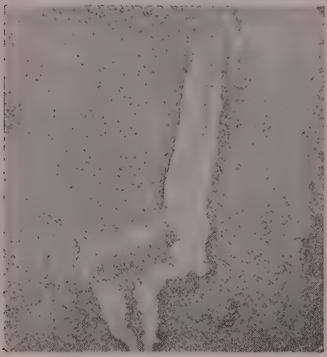

FIG. 3.
Uterus of a rat during pseudo-pregnancy induced by suckling. Normal endometrium on the right, large placentoma invading the uterine cavity from the left.

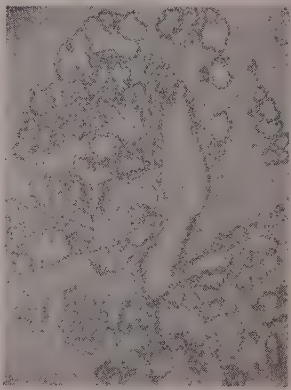

FIG. 4.
Secreting mammary gland with distended alveoli in a rat weaned for a short period, and then given litter again.

tain investigators, suggesting a hormonal basis for maternal instincts, it seemed unlikely that virginal or weaned animals would tolerate the presence of a young litter. But it was found that only rarely would an animal harm the litter, and this occurred no less frequently with lactating mothers than with virgins. In many instances, however, there was no suckling, either from the unwillingness of the animal to allow it or from the inability of the litter to find the relatively small nipples of virginal and weaned rats. Further, nipples frequently became necrotic from the strength of the stimulus, especially in virgins and in animals weaned for a long time.

It was found that the teats could be made more obvious by cutting the hair immediately around them and by teasing them out gently between 2 moistened fingers.

We attempted to expose 6 virgin rats to the stimulus of suckling as described above. Out of this group we found that active suckling occurred only in one case, and in this instance the nipples became necrotic and the animal had to be discarded. Considering that the nipples of a virgin are very much smaller than those of an animal that has at some time been pregnant, we continued our experiments on animals of the latter type.

These animals were taken from large cages in which numerous males and females were kept together. Their previous sex history had not been followed, but their having been pregnant at some time was indicated by the appearance of the nipples. Vaginal smears were taken daily from the day of selection, and only those animals which were regularly cyclic were used.

Of 25 animals given litters, active suckling occurred only in 15 cases. In this group again we observed that some of the nipples became necrotic and for this reason a few animals had to be discarded. There remained, however, a group of 8 animals, in some of which a partial necrosis of a few nipples had been observed in the earlier part of the experiment, but after a few days the teats took on the appearance and flexibility of those of a lactating mother, and from that time the mechanical stimulus of nursing caused no further damage.

These animals became continuously dioestrous, but in some cases an oestrus cycle occurred after the initiation of nursing. As biopsy specimens showed (Figs. 1, 2) the mammary gland developed to the same extent as it would in the course of pseudo-pregnancy. Numerous alveoli appeared around the ducts, but they showed little or no sign of secretion. The uteri became progestational in appearance.

In many cases large placentomata were found (Fig. 3). The ovaries contained one set of large corpora lutea similar to those of pregnancy. Maturing follicles were never observed. The average weight of the ovaries was about 80 mg., and that of the pituitaries 16 mg. These weights correspond closely to those that we find in the pregnant animals of our colony. In some cases silk threads were placed in the uterus, and the placentoma development typical of pseudo-pregnancy followed.

These findings lead us to the conclusion that the stimulus of nursing induced a condition resembling pseudo-pregnancy.

In the next series, a group of 5 rats was weaned on the third day postpartum, from which time vaginal smears were taken. A litter was given to 2 of these after the appearance of the first cycle, and to the other 3 after the appearance of the second cycle. Although the mammary glands had stopped secretion and had undergone severe atrophy in every case at that time, secretion started again in all animals under the influence of suckling (Fig. 4). Further, immediately on the return of the litter the cycles stopped and dioestrous set in, lasting 13 to 16 days, except in the case of one animal which was still dioestrous on the twenty-second day, at which time it was killed.

From these experiments it appears that mechanical irritation of the nipple may lead to a condition closely resembling, if not identical with, the pseudo-pregnancy produced by sterile copulation. It is interesting to note that this same stimulus which maintains secretion in the mammary gland of the normal lactating animal leads to the development of glandular epithelium similar to that seen in pregnancy, but with little or no secretion when applied to an animal in which the mammary gland has undergone involution.

In view of these findings the question arises whether the corpus luteum of lactation is not merely a corpus luteum of pseudo-pregnancy produced by the act of suckling. Although some workers have reported that placentomata may be produced during lactation in rodents by irritation of the uterine mucosa,[3] we ourselves have rarely seen spontaneous deciduomata at this period.

Considering that almost all of our experimental animals showing pseudo-pregnancy also showed spontaneous placentomata, we feel that there must be a difference in the response of the animal to suckling, depending upon whether the stimulus is applied during or outside of lactation. This view is further substantiated by the fact that no obvious mammary growth is observed during lactation, but secretion is active, while during pseudo-pregnancy the growth processes are very conspicuous, and little or no secretion occurs.

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[3] Corner, E. W., and Warren, S. L., *Anat. Rec.*, 1919, **16**, 168.

Summary. A condition resembling pseudo-pregnancy has been obtained in adult female rats, by the mechanical irritation of the nipple, produced by an actively suckling litter.

7277 P

Dependence of Continuous Bioelectric Currents upon Cell Oxidation.

H. F. ROSENE. (Introduced by E. J. Lund.)

From the Department of Zoology, University of Texas.

Lund has shown that bioelectric potentials are quantitatively linked with oxidations (frog skin, hydroids, etc.) and has advanced the theory that continuous bioelectric currents are established by redox systems in flux equilibrium within the cell.[1] The new findings

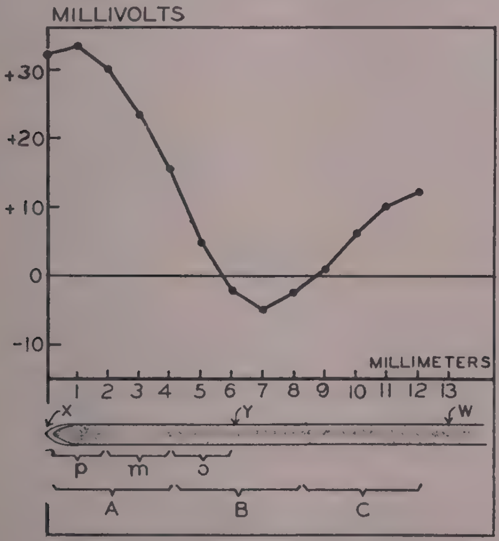

Fig. 1.

Typical curve of distribution of E. M. F. per unit length of root *(A. cepa)*. Abscissa, position of positive electrode in millimeters from the tip. Root regions are drawn to scale. See text for further description.

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[1] Lund, E. J., *Protoplasma*, 1931, **13**, 236.

have a significant bearing on (1) the nature of the electrochemical mechanism which generates a continuous output of electric energy in living cells, (2) the nature of the specific differences in the oxidative metabolism of young and old tissues, and (3) the rôle of electric energy in cell division, cell correlation, growth, and the absorption of water and solutes.

The curve in Figure 1 shows the typical distribution of E.M.F. per unit length of root in the onion *(Allium cepa),* in the uninjured unstimulated condition. The region of active cell division, p, maintains a high positive potential with respect to all other regions. Ordinarily, at a distance 5 to 12 millimeters from the extreme tip, there is a region of relatively low positivity followed by a region of increased positivity. Fluctuations in electric polarity, which are not due to any observable change in the environment, occur, and the distribution of E.M.F. varies from time to time.

The major facts which show that the magnitude and orientation of bioelectric currents in the onion root depend uniquely upon the concentration of oxygen and therefore upon the respiratory mechanism of the cell are as follows:

1. When electrodes using the native medium for contact, are placed at the positions X and Y then: (a) Removal of oxygen around the zone of active cell division, p, greatly decreases the E.M.F. of the root between X and Y. This decrease frequently inverts the electric polarity and in all experiments the potential remains at a relatively low level. Removal of oxygen around the zone of cell maturation and elongation, m, also produces a definite but less marked decrease in E.M.F. of the region X-Y. A similar small decrease in E.M.F. occurs when the region, o, in which permanent tissues are present, is deprived of oxygen. (b) Upon admission of pure oxygen arond zone, p, after a period in its absence, an abrupt increase in E.M.F. occurs and the potential invariably exceeds its initial value. Reversible effects are also obtained when the regions, m, and o, in different roots are similarly exposed to oxygen after a period in its absence, but the rise in E.M.F. is less, and the conspicuously large increase over the initial value does not appear. (c) When all the different regions, p, m, and o, are simultaneously deprived of oxygen, a large decrease in the E.M.F. occurs, demonstrating the unequal effect of equal change in oxygen tension on the specific regions between X and Y. Measurements of E.M.F. per unit length show that in the absence of oxygen, the region of active cell division, p, becomes electronegative to the regions, m, and o, changing the characteristic pattern of correlation currents and

decreasing the total output of electric energy by the root, as measured. (d) The simultaneous addition of pure oxygen, after its absence, to all the regions, p, m, and o, produces a sharp drop in the total potential of the length X-Y, followed by a relatively enormous increase, frequently trebling the initial value. Distribution measurements show that in each root, the region, p, becomes highly electropositive to other regions, and the E.M.F.'s of the specific regions between contacts X and Y are each relatively greater in magnitude than in any previous period.

2. When electrodes are placed at the positions X and W, 13 millimeters above X, then: (a) A *drop* in the total E.M.F. of the length X-W is observed when oxygen is removed from the region indicated by the bracket A. On the other hand, a *rise* in the total E.M.F is observed upon the removal of oxygen around the region designated by C. Variable results are obtained when oxygen is removed around the region B The fact that the removal of oxygen around regions which exhibit oppositely oriented electric polarities produce opposite effects on the polarity of the whole furnishes confirmatory evidence of the principle of algebraic summation of cellular E.M.F.'s.

3. When electrode contacts are made at X and Y, and the region C, outside of the contacts, is exposed to absence and presence of oxygen, no change in the total E.M.F. of the length X-Y appears. Neither is a change in the total potential observed when the contacts are made at Y and W, and the region A is exposed to the same change in oxygen concentration.

7278 C

Glutamic Acid in Milk Anemia. Effect on Hemoglobin Regeneration in "Cystine Deficient" Animals.*†

ERWIN BRAND AND CHARLES J. STUCKY.

From the Department of Chemistry, New York State Psychiatric Institute and Hospital, New York City.

Certain investigators[1] have reported that glutamic acid supplements Fe in the treatment of milk anemia in rats, but this finding has not been confirmed by others.[2, 3] In our experiments[4] carried out with normal rats from our stock colony the addition of glutamic acid to various metals did not produce any supplementary effect on Hb regeneration and growth.

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* In cooperation with the Research Department of the Calco Chemical Co., Bound Brook, N. J.

† This paper was presented in part before the Society of American Biological Chemists, Philadelphia, Pa., 1932.

[1] Drabkin, D. L., and Miller, H. K., *J. Biol. Chem.*, 1931, **90**, 531.

Certain observations,[5] however, appeared to indicate that factors other than the composition of the milk ration and the supplements *per se* might play a rôle in Hb regeneration. Accordingly the following experiments were undertaken.

A group of 8 rats which had been on a deficient diet was selected. These animals had received for approximately 3 months a ration of the following composition: corn starch 54.5, lard 24.5, casein 9, salt mixture (O.M.) 4.5, sugar 4.5, and cod liver oil 3%.[6] It should be noted that this diet is low in protein and deficient in cystine. This ration was continued for an additional 2 months, supplemented by fresh green lettuce (approximately 20 gm. per rat daily). At the end of this period the rats were bred (normal males were used). The fertility of the animals was found to be low and the mothers had difficulty in nursing their young properly. During the lactation period the mother rat received about 1 oz. of liquid milk per day in addition to the diet. When the pups were 18 days old, whole milk powder (klim) was substituted for the low protein (cystine-deficient) diet above. The pups were weaned when 22 to 25 days old and weighed 22 to 37 gm. Only 3 litters with a total of 11 animals were weaned successfully by the above procedure.

For the production of anemia the procedure previously described[4] was employed. The blood Hb level was reduced to approximately 4 gm. per 100 cc. in 3 to 7 weeks, except in one case in which it required 94 days to produce anemia.

The details of the experiment are shown in Table I. Five animals from 3 litters were fed 0.25 mg. of Fe. None of these rats recovered from the anemia and all of them died before the expiration of the 8th week. An attempt to save some of the animals by transferring them to other supplements was unsuccessful. Three animals were fed a larger dose of Fe (.5 mg.). Two of these rats

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[2] Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1931, **93**, 197.

[3] Keil, H. L., and Nelson, V. E., *J. Biol. Chem.*, 1932, **97**, 115.

[4] Brand, E., and Stucky, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 627.

[5] Stucky, C. J., and Brand, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 932.

[6] Vitamin supplements: 200 mg. of vitavose + 50 mg. of yeast concentrate or 150 mg. of dried yeast.

died within a few weeks. The 3rd (Rat 651 of litter 91Y) regenerated, but it grew very slowly (weight 126 gm. at the end of the 12th week). Since 7 of the 8 animals which received Fe supplements (either at the .25 mg. or .50 mg. levels) failed to regenerate

TABLE I.

The Effect of Fe and of Glutamic Acid on Hb Regeneration in "Cystine Deficient" Milk Anemic Rats.

Litter No.	Rat No.	Supplement*	weeks on supplement		Hemoglobin	
					Initial	Final
89W	642 ♂	.25 mg. Fe + 70 mg. glutamic acid	12	a[1]	4.0	11.1
				b[2]	.28	1.32
	643 ♀	.25 mg. Fe	a†	a	3.9	3.2
				b	.20	.15
90YW	644 ♂	.25 mg. fe + .01 mg. Cu	12	a	3.5	14.2
				b	.20	1.52
	645 ♂	.25 mg. Fe + 70 mg. glutamic acid	12	a	4.4	12.4
				b	.27	1.40
	646 ♂	.5 mg. Fe	1†	a	3.2	2.1
				b	.15	.10
	647 ♂	.25 " "	5†	a	3.6	3.8
				b	.22	.16
	648 ♀	.25 " "	2†	a	3.7	2.7
				b	.20	.15
	649 ♀	.25 " "	6†	a	4.7	3.0
				b	.28	.16
91Y	650 ♂	.25 " "	3†	a	3.3	2.2
				b	.24	.12
	651 ♀	.5 " "	12	a	3.3	12.3
				b	.16	1.02
	652 ♀	.5 " "	3†	a	2.9	2.0
				b	.15	.10

* The Fe was fed as $FeCl_3$ and the Cu as $CuSO_4$.

† Animal died.

a[1] = Hb in gm. per 100 cc.

b[2] = "Total Hb" in gm., *cf.* Stucky, C. J., *J. Biol. Chem.*, 1932, **97**, XIII.

and died, the result obtained with Rat 651 is perhaps due to individual variation.

On the other hand, the 2 rats (642 and 645) which received .25 mg. of Fe supplemented by 70 mg. of glutamic acid were cured of their anemia and showed good growth, attaining a weight of approximately 200 gm. by the end of the 12th week. A control animal (644) which received Fe+Cu showed more rapid Hb regeneration but the growth was slightly less than that of the rats which received glutamic acid.

In the above experiment we used the same milk (klim), Fe and glutamic acid preparations as in our recent experiments with normally bred animals from our stock colony.[4] The "cystine deficient" animals died on Fe supplements alone but recovered from their anemia when glutamic acid was added to the iron, while the "normal" animals survived on Fe supplements and the addition of glutamic acid was without effect.

The number of "cystine deficient" animals was necessarily small, due to the difficulties in breeding and, therefore, no definite conclusions can be drawn. Still it would appear suggestive that, under conditions, glutamic acid may have an hematopoietic effect.

Differences in the animals themselves may be an important consideration in interpreting the discrepancies in the findings of various laboratories.

Secondary anemias and particularly milk anemia in the rat may frequently appear to be only mineral deficiencies, but, under conditions, this simple picture may be complicated by other maladjustments which in turn will determine aspects of hematopoiesis and of therapy.

Summary. Rats bred from mothers subsisting on a low protein (cystine-deficient) ration recovered from their "milk anemia" and showed good growth when glutamic acid (70 mg.) was added to their iron supplement; whereas, those from which glutamic acid was withheld but the iron added, died. The significance of this finding has been indicated.

7279

In vitro and in vivo Cultivation of the Virus of Rift Valley Fever.

R. S. SADDINGTON.

From the Laboratories of The Rockefeller Institute for Medical Research.

That viruses may be cultivated successfully through many generations is being increasingly found as new ones are being submitted to the process of cultivation. The method now widely employed for securing cultures *in vitro* is that devised by Li and Rivers[1] for vaccine virus. A similar device introduced by Goodpasture,[2] of the chorio-allantoic membrane of the developing chick, may be regarded as an example of cultivation *in vivo* quite distinct from the ordinary multiplication which occurs in the course of usual infection in developed, susceptible animal species. Just as it had previously been shown that herpes virus grown in tissue culture and in chick embryo may be titrated in the brain of mice,[3] it has now been shown that it is possible to cultivate and titrate, in the ways indicated, the virus of Rift Valley fever of sheep.

Dr. G. M. Findlay kindly supplied the material with which the cultivations were begun. It consisted of the liver of a mouse in the 86th passage. We owe indeed to Dr. Daubney and Dr. Findlay[4] the discovery of the virus nature of Rift Valley fever.

In vitro Cultures. The medium employed consisted of Tyrode solution to which minced 10-day-old chick embryo was added. The Rivers' flasks contained 4.5 cc. of this medium, and to each was added 0.2 cc. of a Berkefeld filtrate of a 10% phosphate buffer emulsion of infected mouse liver. The titration was made by inoculating 0.2 cc. of a dilution of a culture intraperitoneally into mice. Two series of cultures, I and II, were run. Series I was carried through 11 and Series II through 12 generations.

The results of the inoculations indicate (a) that the optimum incubation period for the cultures was from 4 to 5 days; (b) that intraperitoneal injection of the cultures gave more uniform results than intracerebral, intramuscular, intratesticular, or intradermal inoculations; and (c) that both Seitz and Berkefeld N filters keep back large amounts of the virus.

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[1] Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465; Rivers, T. M., *idem.*, 1931, **54**, 453.

[2] Woodruff, A. M., and Goodpasture, E. W., *Am. J. Path.*, 1931, **7**, 209; Goodpasture, E. W., Woodruff, A. M., and Buddingh, G. J., *Science*, 1931, **74**, 371; Rivers, T. M., and Schwentker, F. F., *J. Exp. Med.*, 1932, **55**, 911.

[3] Saddington, R. S., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1012.

[4] Daubney, R., Hudson, J. R., and Garnham, P. C., *J. Path. and Bact.*, 1931, **34**, 543; Findlay, G. M., and Daubney, R., *Lancet*, 1931, **2**, 1350; Findlay, G. M., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1932, **25**, 229.

In vivo Cultures. The usual technique was employed. The inoculum consisted of 2 drops of a Berkefeld filtrate of a 10% saline suspension of mouse liver. The inoculation was made into the chorio-allantoic membrane of 9 to 10-day-old chick embryos. Three series were run, the largest consisting of 5, and the smaller of 3 and 2 passages respectively. Examination of the embryos was made after 5 days of incubation following the inoculation, and the subsequent inoculations were made with (a) membrane, (b) liver, (c) amniotic fluid of the embryos. Titrations were carried out in mice.

The inoculated chick embryos showed greyish discoloration or a gelatinous swelling of the embryo, and yellow or yellow-white mottling or stippling of the liver. The liver, membrane, and amniotic fluid all contained virus, although not constantly. The liver was more regularly infectious for mice than the other materials employed for inoculation.

Pathological Histology. Chorio-allantoic chick membranes. Certain areas of epithelial cells show evidences of hyperplasia, while other areas consist of necrotic cells. The adjacent connective tissues are undergoing inflammatory reactive changes; while the livers of embryos exhibit focal or widespread necrosis, the latter involving most of the organ. Nuclear inclusions were not found in the membranes or organs of the embryos.

Chick embryo tissue. Fragments of the tissues used in the *in vitro* cultures were sectioned and stained. Necrotic and living cells lie side by side. No nuclear inclusions are seen.

Mouse livers. The livers of the inoculated mice succumbing to infection show extensive degenerative lesions similar to those resulting from mouse to mouse passages of the virus. The lesions are so extensive that cords of hepatic cells are reduced to fragments or wholly destroyed, their places being taken by dilated blood spaces. Acidophilic inclusion bodies are present in the liver cells.

In view of the amount of dilution produced by the successive subcultures, there is little doubt that it has been shown that the virus of Rift Valley fever of sheep is subject to cultivation outside the body of infected sheep and mice.

7280 P

Distribution of Virus of Louping-Ill in Blood and Brain of Intranasally Infected Mice.

G. L. FITE AND L. T. WEBSTER.

From the Laboratories of The Rockefeller Institute for Medical Research.

Mice given an intranasal instillation of louping-ill virus, following an incubation period of 6-7 days, regularly develop signs of encephalitis and die 8-10 days after inoculation with characteristic lesions in the central nervous system.[1, 2] Studies on the presence of the virus in blood and brain of intranasally inoculated mice will now be reported.*

White-face and Swiss mice have been inoculated by dropping into the nose 0.03 cc. of a 1 to 10 suspension in physiological salt solution of the brains of mice dying of louping-ill. The virus content of the blood has been studied in 2 ways. In one experiment mice were inoculated and bled twice daily from the tail for 8 to 9 days—that is, until dead. One or 2 drops of blood were collected in 0.25 cc. of a solution of heparin, 1-1,000 in salt solution, and 0.03 cc. of this was inoculated into each of 2 other mice. In the second case, 36 mice were inoculated and 4 were killed daily, the whole blood being withdrawn directly from the heart and inoculated immediately into 2 mice. The mice were then killed, the brains removed and emulsified, and this suspension was also inoculated into other mice.

The results of these experiments showed that virus did not appear in the blood stream of nasally infected mice in quantity until the 2nd day after inoculation. From then on to the 6th day, when the mice became sick, the virus was usually demonstrable in the blood. After the 6th day, until the animals were moribund, the virus tended to disappear. The brains of these mice occasionally contained demonstrable virus as early as the first day but in 50% of cases not until the 3rd day. After the 3rd day the virus was invariably present in large amounts.

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[1] Webster, L. T., and Fite, G. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 656.

[2] Elford, W. J., and Galloway, I. A., *J. Path. and Bact.*, 1933, **37**, 385.

* Greig, Brownlee, Wilson and Gordon[3] and MacLeod and Gordon[4] have demonstrated the virus in the blood stream of experimentally infected sheep.

[3] Greig, J. R., Brownlee, A., Wilson, D. R., and Gordon, W. S., *Vet. Rec.*, 1931, **11**, 253.

[4] MacLeod, J., and Gordon, W. S., *J. Comp. Path. and Therap.*, 1932, **45**, 240.

To correlate the appearance of virus in blood and brain of nasally infected mice with the appearance of lesions in the central nervous system, groups of white-face mice were inoculated intranasally and 4 killed each day for 8 days. The brains and cords were sectioned and stained for study. Definite lesions were not observed before the 5th day, at which time they were not extensive. On the 6th and 7th days, however, the lesions were fully developed.

It appears, therefore, that louping-ill virus introduced into the noses of susceptible mice gains access to blood and brain and is demonstrable in both by the 2nd day. It persists in the blood until cortical lesions develop and the animal becomes ill, at which time it tends to disappear. Virus is present in the brain at least 4 days before the animal appears sick and persists and multiplies there until the animal succumbs to encephalitis.

TABLE I.

Relation of Presence of Louping-Ill Virus in Blood and Brain of Intranasally Inoculated Mice to Appearance of Central Nervous System Lesions and Course of Disease.

Remarks	Days after Intranasal Inoculation									
	1	2	3	4	5	6	7	8	9	10
Virus in blood	0	+	+	+	+	±	±	±	±	±
” ” brain	±	+	+	++	++	++	++	++	++	++
Lesions in central nervous system	0	0	0	0	±	+	++	++	++	++
Signs of disease	0	0	0	0	±	+	++	++	++	++

7281 P

Ultra-Filtration Experiments with the Encephalitis Virus from the St. Louis Epidemic.

JOHANNES H. BAUER, GEORGE L. FITE AND LESLIE T. WEBSTER.

From the Laboratories of the International Health Division of the Rockefeller Foundation, and the Rockefeller Institute for Medical Research, New York.

Webster and Fite[1] showed recently that the virus isolated from cases of encephalitis in the St. Louis and Kansas City epidemics of 1933, passes through Berkefeld N candles in a relatively high concentration. While these findings indicate that the virus particles are exceedingly small, it was considered of interest to determine their size more accurately by filtration through graded collodion membranes of known porosity.

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[1] Webster, L. T., and Fite, G. L., *Science*, 1933, **78**, 463.

The membranes used in these experiments were prepared according to the method of Elford[2] with certain minor modifications adopted by Bauer and Hughes.[3] They were sterilized by steaming for one hour at 100° C. The average pore values were calculated according to Poiseulle's law from data obtained by measurement of the thickness of the membrane, its water content, the rate of flow of water through a known area, and the pressure producing the flow. These measurements were made after the sterilization.

The virus-containing material used consisted of fresh brain tissue from mice which had succumbed to the experimental disease. In most of the experiments 4 brains were ground in a sterile mortar, suspended in 100 cc. of a diluent of equal parts of hormone broth (pH 8.0), ascitic fluid, and distilled water. The suspension was centrifuged for 30 minutes at about 3000 r.p.m. The supernatant fluid was passed through a Seitz filter, and then through a collodion membrane with an average pore diameter of about 0.25μ. This stock filtrate was then passed through a series of membranes of different pore sizes. From 6 to 8 different membranes were used in each experiment. The effective filtration area for each membrane was about 5 sq. cm., and the amount of the virus suspension passed through such area was usually 10 cc. To reduce the amount of adsorption of proteins from the virus suspension by the membranes, 3 cc. of sterile broth was passed through each membrane before filtration of the virus suspension. All filtrations were carried out under applied positive pressure of nitrogen. The amount of pressure used in most of the experiments was 100 cm. mercury, and only in one experiment, in which membranes of the finer grade were used, were three atmospheres applied. In no case was there observed any stretching or damage to the membranes.

Each filtrate was tested in a group of 6 mice, and the amount injected into each mouse was 0.03 cc. intracerebrally. The stock filtrate in each experiment was titrated in mice to a dilution of 1:100,000, and in most of the tests was found to be infective in this dilution.

Nine filtration experiments were carried out, using a total of 25 membranes of different porosities. These experiments are summarized in Table 1.

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[2] Elford, W. J., *J. Path. and Bact.*, 1931, **34**, 505.

[3] Bauer, J. H., and Hughes, T. P., to be published shortly.

TABLE I.

Summary of ultra-filtration experiments carried out with the encephalitis virus.

No. of Membrane	Aver. pore diam. m. μ	Thickness membrane mm.	No. filtration exper.	No. times filtrate infective
165	257.7	.15	1	1
157	218.8	.13	1	1
137	162.3	.15	1	1
99	121.3	.18	1	1
107	100.0	.22	1	1
87	90.8	.17	4	4
86	82.6	.17	2	2
189	72.3	.16	2	2
89	71.2	.18	2	2
213	69.3	.17	2	2
160	67.6	.14	4	1
77	67.0	.20	4	4
186	66.9	.16	2	2
139	66.1	.11	6	3
184	62.5	.16	2	None
208	59.7	.14	1	,,
91	57.0	.19	4	,,
149	55.7	.17	2	,,
151	54.8	.17	1	,,
202	51.8	.13	2	,,
79	51.8	.17	2	,,
131	49.3	.14	4	,,
132	43.0	.14	2	,,
114	38.8	.16	1	,,
153	28.0	.13	2	,,

As seen from this table, the virus passed consistently through all membranes with an average pore diameter greater than 66 millimicrons, while membranes with pore diameter smaller than this gave uniformly negative results. However, the amount of the virus passing through membranes between 72 and 66mμ. appeared to be greatly diminished. The strain of mice used in these experiments is highly susceptible to the virus, and the titrations of the stock filtrates almost invariably produced 100% mortality in the mice inoculated with dilutions up to 1:10,000. On the other hand, the mortality rate among the mice inoculated with the filtrates of the membranes between 72 and 66mμ. was usually 4 or 5 in a group of 6. Of the 6 experiments carried out with membrane No. 139, which has a calculated average pore diameter of 66.1mμ. three gave entirely negative results, in one experiment only 2 of the 6 mice died, and in the remaining 2 experiments all mice died. The filtration end point, in these experiments, appears to be about 66 millimicrons.

All collodion membranes have a strong adsorptive affinity for proteins, and in filtration experiments usually little protein passes through a membrane before this affinity is satisfied. This adsorption naturally reduces the diameter of the pores, the extent being dependent on the amount of cohesion of protein molecules taking place in the capillary channels of the membrane. In view of this phenomenon, Elford[4] has estimated that in filtration experiments with membranes of greater porosity than 10mμ, a particle must have a diameter only from one-half to one-third of that of the pore in order to traverse it. The application of this formula to the above experiments would indicate that the probable size of the encephalitis virus lies somewhere between 22 and 33 millimicrons.

7282 P

Observations on Induction of Anaphylactic Shock by the Specific Carbohydrates of Type-I Pneumococcus.

RACHEL BROWN.

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Further comparative studies of the soluble specific substance of Avery and Heidelberger and of the specific "cellular carbohydrate"[1] by means of the experimental induction of anaphylactic shock have given additional information regarding the antigenic activities of these preparations. Avery and Tillett[2] induced fatal anaphylactic shock in passively sensitized guinea pigs with the homologous soluble specific substance of the pneumococcus, and Enders[3] reported that antiserum, after precipitation with soluble specific substance, still sensitized guinea pigs to his "A" substance but not to the soluble specific substance. This report records experiments with the type-I specific substances injected intracardially into guinea pigs which had been passively sensitized with type-I antipneumococcus rabbit serum 24 or 48 hours previously.

The minimum amount of immune serum necessary to sensitize guinea pigs so that fatal shock developed when they were injected intracardially with soluble specific substance was always greater than that required in the case of the cellular carbohydrate. On the other hand, the minimum amount of each of the specific substances which induced fatal shock in the guinea pig previously sensitized by the injection of 1 cc. of immune rabbit serum was approximately the same.

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[4] Elford, W. J., *Proc. Roy. Soc. B.*, 1933, **112**, 384.

[1] Wadsworth, Augustus, and Brown, Rachel, *J. Immunol.*, 1933, **24**, 349.

[2] Avery, O. T., and Tillett, W. S., *J. Exp. Med.*, 1929, **49**, 251.

[3] Enders, J. F., *J. Exp. Med.*, 1930, **52**, 235.

A subcutaneous injection of 1 mg., but not 0.1 mg., of soluble specific substance 24 hours after sensitization desensitized the guinea pigs completely to a subsequent intracardial injection (24 hours later) of soluble specific substance, but not to an injection of cellular carbohydrate. One mg. of the latter, however, desensitized entirely to the soluble specific substance and partially (no fatal shocks) to the cellular carbohydrate.

When the soluble specific substance of Avery and Heidelberger was added to the minimum dose of antipneumococcus serum necessary to sensitize and the mixture injected immediately into guinea pigs, the animals were not sensitized to a subsequent intracardial injection of this substance but were to the cellular carbohydrate. On the other hand, when the cellular carbohydrate was substituted for the soluble specific substance in this experiment, no sensitization was demonstrated toward either the soluble specific substance or the cellular carbohydrate.

In the foregoing experiments, the entire mixture of serum and carbohydrate was injected. In previous experiments,[1] in which the precipitates were removed, the supernatant antiserum, after precipitation by the soluble specific substance, sensitized the guinea pig to the cellular carbohydrate but not to the soluble specific substance. Antiserum, after precipitation by the cellular carbohydrate, however, failed to sensitize the guinea pig to either substance.

The results of these experiments correspond with those previously reported and record additional data concerning the broader activities of the cellular carbohydrate as compared with the soluble specific substance of Avery and Heidelberger.

7283 P

Failure of Antipneumococcus Horse Serum to Sensitize Guinea Pigs to Anaphylactic Shock with Specific Carbohydrates.

RACHEL BROWN.

From the Division of Laboratories and Research, New York State Department of Health, Albany.

Previous experiments have demonstrated noteworthy differences in antipneumococcus sera from the horse and from the rabbit. For example, immune horse serum, in striking contrast to immune rabbit serum, intensifies the purpuric activity of the type-I pneumococcus cellular carbohydrate in mice.[1] Also, these 2 sera behave very differently with respect to the fixation of complement in the presence of specific carbohydrates[2] and precipitation in the presence of the partial hydrolysis products of type-III soluble specific substance.[3] Finally, marked differences have been reported[4, 5] in the passive sensitization of the guinea pig to the soluble specific substance by immune horse and rabbit sera; the latter sensitizes, while the former does not. Evidently there are fundamental differences in the action of the sera of the horse and the rabbit immunized with the pneumococcus, concerning which it is important to obtain further information.

In the experiments here summarized, type-I antipneumococcus horse serum, alone or in combination, was used as the sensitizing agent and the homologous soluble specific substance or the cellular carbohydrate as the test material for sensitization. The observations of others have been limited to the soluble specific substance, but in these experiments similar results were obtained with both carbohydrates. The sensitizing dose was given to guinea pigs intraperitoneally and the test dose intracardially 24 or 48 hours later.

The problem of passive sensitization with antipneumococcus horse serum was considered from the point of view that this serum either lacked a factor necessary for the sensitization of guinea pigs or contained an inhibiting substance.

The first group of experiments demonstrated that type-I antipneumococcus horse serum did not become a passive sensitizing agent for anaphylactic shock when mixed with normal rabbit serum, antisheep amboceptor produced in the rabbit, heterophile antibody, or with the sera of rabbits which had been inoculated with any one of the following: type-I antipneumococcus horse serum, the soluble specific substance, the cellular carbohydrate or the ether-soluble fraction of the pneumococcus. Thus, none of these added sera (inactive in themselves) was capable of supplying a missing factor in the horse serum.

The second group of experiments was divided into 2 series. From the first series it was shown that the sensitizing action of minimal doses of type-I antipneumococcus rabbit serum was not affected by the addition of normal or type-I antipneumococcus horse serum. In the second series neither inactivation of type-I antipneumococcus horse serum at 56°C. nor treatment with the homologous bacterial cells or agar rendered the serum capable of passively sensitizing guinea pigs. These experiments, therefore, failed to demonstrate any inhibiting action of the immune horse serum.

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[1] Wadsworth, Augustus, and Brown, Rachel, *J. Immunol.*, 1933, **24**, 349.

[2] Avery, O. T., and Heidelberger, Michael, *J. Exp. Med.*, 1925, **42**, 367.

[3] Heidelberger, Michael, and Kendall, F. E., *J. Exp. Med.*, 1933, **57**, 373.

[4] Avery, O. T., and Tillett, W. S., *J. Exp. Med.*, 1929, **49**, 251.

[5] Mehlman, Julia, and Seegal, B. C., *J. Immunol.*, 1934, **26**, 1.

A further attempt to activate the serum by a combination of the above methods, in which normal rabbit serum was added to type-I antipneumococcus horse serum, after treatment with a suspension of pneumococci (type-I) and centrifugalization, was likewise unsuccessful.

7284 C

A Note on the Spread of Poliomyelitis Virus in Monkeys.

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Jungeblut and Spring[1] transected the spinal cords of 2 monkeys at the level of the first lumbar vertebra and then injected them intracerebrally with poliomyelitis virus. One animal developed a condition which might have been poliomyelitis, but when separate emulsions, made from the upper and lower parts of the cord, were injected into other monkeys, the disease was not reproduced. Postmortem autolysis prevented a positive histological diagnosis. The second animal developed poliomyelitis. Histologically, there was an absence of lesions considered typical for poliomyelitis in sections taken from the lumbar cord, while those obtained from the cervical area were positive. Injections of an emulsion made from the upper or cervical section of the cord of this monkey reproduced the disease, while an emulsion made from the lower or lumbar section did not do so when injected into another animal.

Previously[2] I reported that when the virus of poliomyelitis was injected into the sciatic nerves of Macacus rhesus monkeys, the disease was produced in the cervical area even though the cord had been transected and removed in the region of thoracic X. The only connection between the upper and lower parts of the cord was by way of the sympathetic nerve fibers.

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[1] Jungeblut, C. W., and Spring, W. J., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 1076.

[2] Toomey, John A., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 502.

In these animals, the pathological picture of the distal or lumbar sections of the cord showed complete tissue degeneration, practically an autolysis in all but one specimen as well as a diffuse, but sparse, round cell infiltration and a few leucocytes. There was neither dilatation of nor cuffing about the capillaries. In the one specimen from the lumbar area referred to in the foregoing report, there was slight cellular degeneration, a relative decrease in the number of neurones in the anterior horn cell area and the same diffuse, but sparse, round cell infiltration. There was a lack of capillary dilatation and cuffing. The capillaries in the proximal or cervical sections of the cord were dilated and had cuffing about the vessels. There was also a generalized infiltration of round cells and anterior horn cell degeneration. The cells seen in the cervical cord sections took the contrasting stains, while the whole section from the lumbar cord, save for the lymphocytes present, was a pinkish red.

I agree with Jungeblut and Spring[1] that the spinal fluid plays no essential part in the transfer of the virus along the central nervous system. From my own work, however, I could not understand why, if the virus could travel up the sympathetic nerve fibers from the sciatic nerve to the cervical area, it could not just as well travel by way of the sympathetic system from the upper to the lower part of the cord.

In experiments where the cord is cut and the virus injected intracerebrally, it is obvious that the spread of the virus along the main pathway, *i. e.*, the cord itself, is interrupted, and the virus, in order to reach the lower part of the cord, would have to travel by the somewhat devious route of the sympathetic nervous system, if at all. It was logical, then, to assume that the amount of virus might not be so great in the distal portion of the cord as it would be in the proximal or cervical end. The time element might also play a part, since a sufficient concentration of virus might not have spread to the lumbar area before the animal had died.

The authors[1] correctly inferred that broad conclusions could not be drawn from their experiments and it was thought that further experiments along the same line should be done.

Four monkeys were transected in the region of dorsal IX, and ½ inch of the cord was removed. Transection was made at this height in order to make sure that the virus would not be transferred along the normal nerve fiber connections between the last thoracic and the first 2 lumbar nerves. The animals were then injected intracerebrally with 1.5 cc. of a 2% emulsion of a potent poliomyelitis virus. All 4 monkeys developed tremor and weakness of the muscles of the arms and upper back from 6 to 7 days after the injection. Suddenly, within 24 hours after the weakness and tremor had begun, or 7 to 9 days after the injection of the poliomyelitis virus, all of the animals died so that at first it was not absolutely evident that the animals had poliomyelitis. The abrupt onset of clinical symptoms, with death occurring so soon after, was in contrast with the usual clinical picture seen in monkeys used in other transection experiments where the sciatic nerve had been used for transference of the virus. The latter animals had been injected with virulent poliomyelitis virus and gradually went through all the stages usually seen in monkeys (weighing from 4 to 6 pounds) that develop experimental poliomyelitis, such as furring, tremor, paresis, paralysis, complete quadriplegia and usually death.

Sections were taken from the cervical and lumbar portions of each cord for microscopic examination. Histologically, the cervical sections from all 4 animals were typical enough to make a diagnosis of poliomyelitis and they looked similar to those taken from the same area in animals in which the virus had traveled from the sciatic nerve along the sympathetic system to the upper part of the cord. The lower sections of the cord in 2 specimens looked like sections of the lumbar area previously described herein (after sciatic injection), the cellular elements being nearly autolyzed with the same diffuse, but sparse, round cell infiltration and all the cellular elements, save for the lymphocytes, stained a homogenous pink. There was no dilation of the capillaries, perivascular infiltration or edema.

The histological sections from the lumbar cords of the other 2 animals showed only a relative decrease in anterior horn cells, occasional cuffing, definite capillary increase with dilatation and some sparse, but diffuse, round cell infiltration with an occasional leucocyte present. The cells that were present took the contrasting hematoxylin eosin stain.

Separate emulsions were made from the remaining pieces of the lumbar cord from each of the 4 monkeys and each one was injected into each of 4 other animals. The cervical cord was not tested for pathogenicity since this point was not in question.

For reasons stated previously, it was decided to use a greater concentration of lumbar cord emulsion for the transfer experiments and to inject 2 cc. of a 10% emulsion of poliomyelitis virus intracerebrally for passage transmission of the disease. The amount of lumbar cord remaining after section was only enough to make the required 10% emulsion in one instance. The other 3 animals received the same amount of virus, however, though in a greater dilution of saline. Three of the 4 animals contracted typical poliomyelitis; 2 developed complete quadriplegia and died; one developed weakness of one leg and paralysis of the other and recovered. The remaining animal had furring, but did not develop paralysis.

The 2 animals which developed quadriplegia had been injected with emulsions made from lumbar cords of animals whose sections took the contrasting stains. The others were injected with material made from devitalized lumbar cord emulsions from animals whose sections took the acid pink stain.

Although virus was present in the lower cord, it must have been relatively decreased as compared to the amount present in the upper cord. This is comprehensible when one remembers that, in transecting the cord, the normal blood supply may be disturbed to such an extent that degeneration of nerve fibers of the lower cord would naturally often follow the operation. Since the degeneration of nervous tissue is obvious in some specimens and the area is not completely normal even in the non-degenerated ones, it might be presumed that the absorption of the virus might be so slow that the presence of the small amount that would have been absorbed before the death of the animal would be difficult to demonstrate in passage experiments. It is interesting to point out that after a nerve is sectioned and degeneration of the axis cylinder has set in, absorption of poison ceases.[3] In the experiments herein described, complete degeneration may not have set in, but enough changes may have taken place after these transection operations to accomplish practically the same effect and thus limit absorption of the virus.

It was interesting to note the modification of the clinical picture in the experimental animal brought about by the method of producing the disease as described.

Summary. In transected animals, transfer of the virus can occur from the upper to the lower part of the cord after intracerebral injections of the virus of poliomyelitis as well as from the lower to the upper part of the cord after sciatic injection, presumably along the sympathetic nerve fiber connection.

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[3] Meyer and Ranson, quoted by Zinsser, Hans, in "Resistance to Infectious Disease," Ed. 4, New York, The Macmillan Co., 1931, p. 43.

7285 P

Rôle of Decreased Amount of Gastric Secretion in Production of Pernicious Anemia.

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In addition to the failure to secrete hydrochloric acid, patients with pernicious anemia secrete very little fluid into their stomachs, either while fasting or after the subcutaneous injection of histamin hydrochloride. The average secretion in 32 collections from 5 patients with true pernicious anemia was 20 cc. per hour, as compared with an average of about 150 cc. per hour for normal individuals. To note the part played by the diminished *amount* of secretion, as opposed to a *qualitative* difference from normal, gastric juice from 5 untreated patients with pernicious anemia in relapse (red blood cell counts varying from 1.5 to 3.3 millions per cu. mm.) was collected during a period of 8 weeks and stored in a refrigerator until 1500 cc. had been collected. A daily dose of 150 gm. of gastric juice was incubated with 200 gm. of meat for 2 hours, at a pH of 2.0 and later made pH 5. This was fed for 10 days to a patient with typical pernicious anemia, whose initial red blood cell count was 1.93 million, white blood cells 4200 per cu. mm., hemoglobin 51% (Sahli) 7.14 gm. %. The chart shows that a rise in the percentage of reticulocytes started on the tenth day, and reached a maximum of 9.8% on the thirteenth day. This was accompanied by an increase in the number of red blood cells and in the hemo-

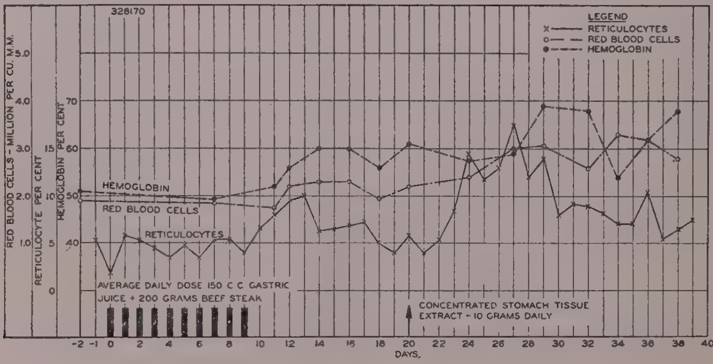

FIG. 1.

globin percentage. From the 10th to the 19th day no further treatment was given and the reticulocyte percentage fell to the pretreatment level. Starting on the 20th day, a daily dose of 10 gm. of a concentrated dried extract of gastric tissue was fed with a subsequent reticulocyte response with a maximum of 17%. The experiment suggests that if pernicious anemia gastric juice, in quantities similar to that secreted by a normal person, is fed (after incubation with beef steak) hematopoietic activity develops. The experiment is now being repeated with various modifications to verify the conclusions.

7286 P

Effect of Ultracentrifuging on Paramecium.

R. L. KING AND H. W. BEAMS. (Introduced by J. H. Bodine.)

From the Zoological Laboratory, State University of Iowa.

The introduction of the air-driven ultracentrifuge described by Beams and his associates[1] affords a means of testing the viscosity and relative specific gravity of many cell components. The authors have been able by the use of this ultracentrifuge to establish the fact that the Nissl bodies of rat ganglion represent a definite material in the cell and are not the result of the action of the fixative used on homogeneous cytoplasm; likewise, for the Golgi apparatus in the uterine gland cells of the guinea pig. In general the nuclei of the cells mentioned above usually show a clearly marked separation of the chromatic from non-chromatic materials in such a way to show that the chromatin is the heavier. The details of this work are to be reported elsewhere. McClendon[2] and Yancey[3] have reported the effect on the division rate of centrifuging various species of Paramecium. McClendon has also described some of the nuclear effects of such treatment.

The ultracentrifuge used in this work was obtained from Professor J. W. Beams of the University of Virginia; the 1⅛ inch rotor was operated by an air pressure of approximately 15 pounds. *Paramecium caudatum* and *P. multimicronucleata* were centrifuged in gum solutions of approximately their specific gravity for varying periods. After 10 minutes most of the animals had disintegrated, a few dead organisms remaining intact. These were dumb-bell shaped (length to width ratio varying from 13:1 to 22:1 compared with the normal 4:1 to 5:1) with prominent knobs on either end. The knob on the centrifugal pole consisted of crystals and the macronucleus, that on the other end of lighter materials of unknown nature. The cytopharynx and contractile vacuoles seemed stretched with the pellicle but otherwise normal in position. In many cases the exploded trichocysts remained in connection with the pellicle. The ratio of length to width in animals centrifuged for 5 minutes varied from 6:1 to 9:1. In such animals the contractile vacuole frequently is still pulsating, the undulating membrane in the cytopharynx and other cilia are still beating. A few of such animals recover. Animals centrifuged for 2½ minutes are more nearly average in bodily proportions (6:1 to 9:1); the body is almost cylindrical, being slightly constricted medianly. The contractile vacuole pulsates and the cilia in general are still active. There also seems to be a greater number of trichocysts in the endoplasmic stream than is the case in the uncentrifuged animals. Single animals isolated after centrifuging for 2½ minutes usually survive and reproduce.

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[1] Beams, J. W., Weed, A. J., and Pickels, E. G., *Science*, 1933, **78**, 338.

[2] McClendon, J. F., *J. Exp. Zool.*, 1909, **6**, 265.

[3] Yancey, Patrick H., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 877.

Paramecium aurelia usually retains its shape longer and survives for a longer period than *P. caudatum* and *P. multimicronucleata,* probably because of its smaller size.

Cytological details were examined in animals fixed immediately after they had been centrifuged for 2½ minutes. They were fixed in Schaudinn's fluid and stained in Hemalum or in Feulgen's sulphurous acid-fuchsin. The crystals had been moved to the centrifugal pole where they form a mass. What appears to be the macronucleus is located next to the mass of crystals.

In some cases the chromatin and crystals have been extruded from the cell leaving an animal of almost normal shape; in still others the whole centrifugal pole seems to have exploded. The pellicle probably prevents a complete cleavage of the organism into 2 fragments as happens in centrifuged Arbacia eggs.

Closer examination shows that, in many cases, of the macronucleus only the chromatin has moved centrifugally; there is left in the usual position of the macronucleus an achromatic, alveolar mass. In other cases, shreds of chromatin material connect the two. An investigation of the history of the recovery of such animals, their reproductive and locomotor features is being carried out.

7287 P

Influence of Optical Activity on Utilization of Lysine for Growth.

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From the Biochemical Laboratory, State University of Iowa.

Recent studies on the relative value for growth of the optical isomers of 2 essential amino acids have yielded divergent results. Thus, *dl*-tryptophane promotes growth as readily as the natural *l* form[1] and the *d* form is likewise as efficient as the *l* form[2, 3]. On the other hand, *i*-cystine is less effective than *l*-cystine[4] and neither *d*-cystine[5] nor one half of the meso form[6] is available for growth. Several years ago McGinty, Lewis and Marvel[7], in studying the replaceability of lysine by related hydroxy compounds, fed control rats both natural (*d*) lysine and synthetic (*dl*) lysine. In the one experiment in which equal amounts of the 2 compounds were employed, the natural amino acid promoted somewhat better growth. As an initial step in evaluating the growth-promoting ability of *l*- and *d*-lysine, we undertook a more exact comparison of the *d* isomer and the *dl* mixture.

d-Lysine was prepared as the dihydrochloride by the method of Cox, King, and Berg[8] and racemized essentially as directed by Fisher and Weigert[9]. The *dl*-lysine was isolated as the dihydrochloride. $[\alpha]_D^{20}$ for the former dihydrochloride (3% solution in water) was +15.64, for the latter 0.0. Analyses for N and Cl agreed with the calculated values. Zein served as the lysine-deficient protein and was prepared essentially according to the method of Brazier[10]. The diet was composed of zein, 14.6; tryptophane, 0.2; cystine, 0.2; starch, 39.5; sucrose, 15.0; Crisco, 19.0; cod liver oil,

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[1] Berg, C. P., and Potgieter, M., *J. Biol. Chem.*, 1931-32, **94**, 661.

[2] du Vigneaud, V., Sealock, R. R., and Van Etten, C., *J. Biol. Chem.*, 1932, **98**, 565.

[3] Berg, C. P., *J. Biol. Chem.*, 1934, **104**, 373.

[4] Lawrie, N. R., *Biochem. J.*, 1932, **26**, 435.

[5] du Vigneaud, V., Dorfmann, R., and Loring, H. S., *J. Biol. Chem.*, 1932, **98**, 577.

[6] Loring, H. S., Dorfmann, R., and du Vigneaud, V., *J. Biol. Chem.*, 1933, **103**, 399.

[7] McGinty, D. A., Lewis, H. B., and Marvel, C. S., *J. Biol. Chem.*, 1924-25, **62**, 75.

[8] Cox, G. J., King, H., and Berg, C. P., *J. Biol. Chem.*, 1929, **81**, 755.

[9] Fisher, E., and Weigert, F., *Ber. chem. Ges.*, 1902, **35**, 3772.

[10] Brazier, M. A. B., *Biochem. J.*, 1930, **24**, 1188.

5.0; salt mixture[11], 4.5; and agar, 2.0%. In each case the lysine supplement replaced an amount of zein. In addition, 200 mg. of yeast was supplied separately to each animal daily. Twelve rats were divided into 4 groups of 3 each. One group received no lysine supplement; the second, 0.125%; the third, 0.25%; and the fourth 0.5%, in the form of an equivalent amount of the dihydrochloride. During the first and third 20 days 2 animals of the second and fourth groups and one of the third received the *dl* modification. The others were fed *d*-lysine. On the second and fourth 20 day periods those animals which had received the *d*-lysine were fed *dl*-lysine, and *vice versa*.

The accompanying table summarizes the findings which indicate that only the dextro component of *dl*-lysine can be used by the rat for purposes of growth. Further studies are in progress.

TABLE I.

Comparative Average Growth of Animals on a Lysine-Deficient Basal Diet Supplemented with *d*- and *dl*-Lysine.*

Rat No., Sex	Aver. daily gain on *d*-Lysine gm.	Aver. daily gain on *dl*-Lysine gm.	Lysine Supplement %
137 ♀	1.20	.60	.5
138 ♂	1.03	.78	.5
144 ♂	.90	.48	.5
Aver.	1.04	.62	
135 ♀	.63	.23	.25
136 ♀	.70	.38	.25
143 ♂	.68	.20	.25
Aver.	.67	.27	
133 ♀	.53	.20	.125
140 ♀	.55	.35	.125
134 ♂	.45	.33	.125
Aver.	.51	.29	
Grand average	.74	.39	

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[11] Hawk, P. B., and Oser, B. L., *Science*, 1931, **74**, 369.

* The average daily growth of the 3 rats on the unsupplemented basal diet was 0.17 gm.

7288 P

Effect of Intravenous Hydrochloric Acid upon Narcosis in Dogs.

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From the Laboratory of Surgical Research, Harvard Medical School

In view of a recent report by MacGilvra,[1] describing a remarkable effect of the intravenous administration of hydrochloric acid as a means of terminating anesthesia, it seemed advisable that further experimental studies be carried out in order to establish more definitely a possible clinical application. MacGilvra, repeating observations made by Moorman, terminated ether anesthesia in rabbits guinea pigs, rats and monkeys by the intravenous injection of small amounts of N/10 hydrochloric acid. MacGilvra reported the clinical application of this method for terminating anesthesia (Palinaesthesia) in a patient under "Avertin."

Our observations were made on 8 dogs, using "Nembutal" intraperitoneally, "Avertin" by rectum and ether by inhalation. It was found impracticable to use chloroform because of the extremely rapid, spontaneous recovery from this drug in the dog. Each observation was made on 2 dogs, simultaneously, one of which was given the intravenous hydrochloric acid. Each pair of dogs was matched, as closely as possible, in weight and breed. Considerable difficulty was encountered in determining the depth of narcosis at the various intervals during the observations, so an arbitrary set of criteria was adopted. In each case an approximately equal degree of deep anesthesia was induced, and the rapidity of recovery gauged by noting the time of appearance of wink reflex, spontaneous movements of the forelegs, attempt to stand, and finally, the ability to stand unassisted. The dose of hydrochloric acid varied from a single injection of 3 cc. of N/10 aqueous solution to repeated injections totalling 65 cc. of the same solution. No untoward effects were noted. The results of our observations are shown in the accompanying table.

It is apparent, that in no case did hydrochloric acid accelerate recovery from anesthesia in the dog. On the other hand, in almost every instance, the animal receiving this medication recovered more slowly than the control. It should be stated, however, that the animal selected for the hydrochloric acid was apparently the deeper anesthetized, so that it was not definitely established that the hydro-

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[1] MacGilvra, W. V., *Harv. Dent. Rec.*, 1934, **8**, 1.

TABLE I.

Dog No.		Wt.	Anesthesia	HCl-N/10	Return Reflexes	Legs Move	Attempt to Stand	Stands
		Kilos		cc.	Minutes			
C25		23.6	Ether	3	(100 sec.)	6	10	15
Z18	C	20.4	”	—	1	4	7	9
Z18		20.4	”	3 3 3	1	4	7	12
C25	C	23.6	”	—	1½	5	9	13
Q32		11.0	Avertin (.125 gm.)	5 20 20 20	25	35	50	60
B343	C	11.4	” ” ”	—	30	35	60	65
Q34		26.9	” (.135 gm.)	5	1	6	10	20
Z7	C	24.4	” ” ”	—	(not abs.)	3	10	15
I179		26.4	Nembutal (.78 gm.)	2 2 2 2 2 2	80	—	—	16 hrs.
I56	C	31.4	” (.98 gm.)	—	80	—	—	6 hrs.

C equals Control.

chloric acid inhibited recovery. Although our observations have shown no effect comparable to that reported by previous observers, further studies on the other laboratory animals are being made.

Pacific Coast Section.

Stanford University Medical School, February 14, 1934.

7289 P

Transmissibility of the Common Cold. Exposure of Susceptible Individuals Under Controlled Conditions.

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The transmissibility of the common cold has been frequently reported in the literature, notably by Dochez and his co-workers, and by Long and others. The following studies were undertaken to investigate this transmissibility under controlled conditions of environment. For this purpose an air conditioned room at the University Hospital was used. This room, which provides bed-space for 4 to 6 patients, is completely isolated from the outside atmosphere, being provided with its own air completely filtered and maintained at any temperature and humidity desired. It contains also an air lock, which permits ingress and egress with a minimum exchange of outside air, and a special lock, which permits service without any break in isolation of subjects. The air-lock, which receives the same air as is supplied to the room, contains the recording and controlling instruments and quarters for the experimental investigator, who was the only one allowed to enter or leave during the course of the experiment.

The room was maintained at a temperature of 70° to 71° F., dry bulb, and 60° to 62° F., wet bulb, which is a relative humidity of 55%. The air flow is approximately 66 cubic feet per minute, which gives a cooling power of 6 to 7 or within the normal limits of Leonard Hill. Barometric pressures were read daily and checked with those of the Weather Bureau. There is a very slight constant positive pressure due to the air being pumped into the room.

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* Supported in part by the Christine Breon Fund for Medical Research and in part by the Herzstein and the Jackson Funds.

The experimental subjects were males between the ages of 21 and 40, who by history had 3 or more colds per year, the average being 5 to 6, and who had not had a cold within recent weeks. They were placed in the room in groups of 3 to 5 and allowed to remain between 5 and 8 days as an incubation period to determine their freedom from an early cold. At the end of this period an individual suffering from a common cold, with onset 12 to 36 hours previously, was brought in and allowed to remain for 24 to 48 hours, the contact being such as exists in normal contacts in a home, namely, eating and playing cards together, use of a common drinking glass, being sneezed at and the like. The experimental subjects were then kept another 4 to 6 days in order to have them under observation during the development of any symptoms. In one group of 5 the degree of exposure was carried to the point of contaminating the thermometers just before temperatures were taken. The material used for this purpose was the fresh nasal secretions which ran freely from the nose of an individual with a common cold in about the twenty-fourth hour, the secretions being allowed to dry on the thermometers. They were obtained from the same individual to whom the subjects were being exposed.

Nineteen subjects in 5 groups of 3 to 4 each were so exposed. No common colds or any symptoms referable to a cold were observed in any of the experimental subjects.

Another experiment performed was the inoculation of experimental subjects with material obtained from individuals suffering from a fresh common cold in the first 24 hours. The method used was to collect the nasal secretion from the individual by letting it drip into a sterile container and then to divide it into 2 portions. One portion was then used without further preparation, the second was centrifugalized at medium speed for 10 minutes, just sufficient to clear it grossly, and the clear supernatant fluid was used. Inoculation of the experimental subjects was carried out by putting 2 or 3 drops of the desired material into each conjunctival sac. Bacteriologically, this method is as effective as intra-nasal inoculation and any direct irritation of the nasal mucous membrane is avoided.

There were 2 groups in this series, the subjects in each group being exposed to a different donor and comprising 5 subjects in one group and 4 in the other. Of these 9 subjects inoculated 4 received the untreated nasal secretions; 4 received the centrifugalized material; one received heated uncentrifugalized material (control).

In no instance did any of these 9 subjects develop any symptoms or signs of a common cold and there were no signs of conjunctival irritation. Five of these subjects had already been exposed to a patient with a cold, and, having developed no symptoms of a cold after an incubation period of 5 days, were then inoculated directly with the nasal secretions of another donor.

Our experiments on a group of 24 presumably susceptible subjects, when kept in an environment arranged for maximum comfort, and exposed to sufferers from a common cold in the early stages, suggest that the common cold is not transmissible under these conditions. We expect to increase the number of subjects exposed and the number of donors to secure results of statistical value. The predisposing factor or factors responsible for the common cold are being investigated.

7290 P

Experimental Encephalography with Anesthetic Gases.*

ROBERT B. AIRD. (Introduced by C. D. Leake.)

From the Department of Surgery, University of California Medical School, San Francisco.

Dogs prepared by previously performed lumbar laminectomies were found most suitable for experimental encephalography studies. Intravenous sodium amytal was given in sufficient amount to quiet the animal so that it could be handled and X-rayed easily, but not in high enough dosage to obscure painful or possible deeper narcotic effects of the anesthetic gases used for encephalographic injection. A lateral skull plate was first obtained. Then the dog was strapped on its side to a special table, the head end of which could be tilted to a 42° angle and which in all positions allowed maximum opportunity both to manipulate and observe the animal's reactions. Next with aseptic technique and local anesthesia a lumbar puncture was performed. The table was elevated and intermittently between collections of spinal fluid the gas to be tested was injected slowly by syringe into the subarachnoid spaces. Rotation of the head aided drainage. A 3-way petcock attached to the injecting syringe and connected by tubing to the controlling valve

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* This work has been conducted under grants from the Christine Breon Fund for Medical Research and the Dudley Cates Fund for Neurological Research in the University of California Medical School.

TABLE I.

		X-Ray Results					Narcotic Effects			After Effects			
Gas	No. Cases	Ventric. Filling	Gas Present	Handling	Danger	Irritation	Sedative	Anesthetic	Recovery	Post-inj. Whine, Struggle	Depression	Hind Leg Weakness or Paresis	Deaths
Air	21	18	7 days	Easy	Safe	20	0	0	—	10	21 (1-3 days)	2	0
Ether	7	?	?	Difficult	Dangerous	7	2	4	10-15 min.	1 (1-?)	0	5	1
Divinyl Oxide	7	5	15-18 min.	”	Very dangerous	6	—	7	10-15 min.	2	0	3	2
Ethyl Chloride	10	5 (2-?)	45 min.	Fairly easy	Very dangerous	10	—	10	20-30 min.	1	0	1-?	1
Vinyl Chloride	5	3 (1-?)	25 min.	Easy	Slightly dangerous	5	?	1	20	0	0	0	0
Nitrous Oxide	5	4	2-3 hr.	”	Safe	1-?	2 (2-?)	0	1-2 hr.	0	0	0	0
Ethylene	7	2 (2-?)	2 hr.	”	”	1-?	4 (1-?)	1	1-2 hr.	1	0	0	0
Acetylene	6	1 (1-?)	30 min.	”	”	2 (late)	2 (2-?)	2	20 min.	4	0	0	0

of the gas tank afforded a simple and convenient closed system for handling the gases. Replacement of the spinal fluid by the gas was carried as far as possible—usually until bubbles returned through the needle. Then the needle was withdrawn and another lateral skull plate was taken. If the ventricular outline appeared, other views were taken to follow the course of the injection. Throughout the entire procedure, pulse, respiration, color, reflexes, tonus, etc., were observed closely and recorded. Repeated encephalograms were obtained on the same animal to compare under as nearly identical conditions as possible the effects of the various gases. Air injections were initially performed in all cases as a standard by which the results obtained with the anesthetic gases could be compared.

With improvements in technique, standardization of routine and experience this method proved quite satisfactory for determining the following information on the various anesthetic gases: (1) ease of handling; (2) early irritative effect; (3) sedative or narcotic effect; (4) after effects, both immediate and late; (5) safety; and (6) X-ray results, including time of absorption.

Using this method, 19 dogs were employed to test the following anesthetic gases: ether, divinyl oxide, ethyl chloride, vinyl chloride, nitrous oxide, ethylene, and acetylene. Special heating coils were used to volatilize the ether and divinyl oxide to body temperature before injection. The results obtained are summarized in Table I. Ethylene and nitrous oxide, because of their non-irritative action, safe sedative effect, lack of after effects and good X-ray results are being studied further.

7291 P

Studies upon Secretory Activity of Glands of the Oral and Pharyngeal Mucous Membranes.*

MARY F. MONTGOMERY. (Introduced by C. D. Leake.)

From the Department of Physiology of the University of Chicago and the Department of Surgery, University of California Medical School, San Francisco.

These studies were undertaken to extend our very limited knowledge relating to the secretory activity of the small mucous and serous glands of the oral and pharyngeal mucous membranes. I have been unable to discover any adequate description of the nerve supply to the various groups of these glands. Therefore, to confirm and to amplify the fragmentary reports of previous workers I determined what nerves carry such secretory fibers by directly stimulating the nerve trunks in 12 anesthetized dogs. I found the innervation to be a dual one through both the sympathetic and cranial autonomic systems, as has been described. Stimulation of the cervical sympathetic trunk in the neck caused secretion homolaterally over all of the gland bearing areas of the oral and pharyngeal mucous membranes. The maxillary division of the fifth cranial nerve sends a branch to the orbital gland, and through its splenopalatine branches supplies the glands of the hard palate, the soft palate, the tonsils, the pillars of the fauces, and the nasopharynx. Of the mandibular division of the fifth cranial nerve, the buccinator branch innervates the orbital gland and the glands of the cheek and lower lip. The lingual branch supplies the glands of the floor of the mouth. Secretory fibers from the glossopharyngeal nerve reach the glands at the base of the tongue and the posterior fourth of the side of the tongue, the hard and soft palates, the tonsil, the pillars of the fauces, and the nasopharynx. The vagus sends fibers to the nasopharyngeal and epiglottic glands, and to the glands at the base of the tongue over a small area near the middle line.

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* This work has been conducted in part under a grant from the Christine Breon Fund for Medical Research in the University of California Medical School.

I believe these small glands do not secrete continuously, but only reflexly in response to an adequate stimulus as is the case for the salivary glands. Studies upon 5 intact, unanesthetized dogs and 4 human subjects have shown that the glands of easily observed areas, namely, the dog's lower lip and cheek and the human lips and palate, are reflexly excited by a large number of different stimuli, among which are pressure upon the tongue or palate, licking and swallowing movements, the odors of acetic acid, ammonia, and ether, and the taste of salt, sugar, hydrochloric and acetic acids, and magnesium sulphate. By observing the lower lips of resting dogs for periods of 30 minutes I demonstrated that in the absence of any stimulation, no secretion occurred during this interval, but as soon as licking and swallowing movements were induced, the glands secreted promptly and copiously. Other areas were not studied because they could not be observed without mechanically stimulating the mouth. I obtained additional indirect evidence on the absence of a continuous secretion from 8 dogs without parotid, submaxillary, sublingual, and orbital glands, the remaining oral and pharyngeal secretions of which were collected from an esophageal fistula. In such dogs, when at rest, each stimulation of the esophageal stoma and resulting swallowing movement expelled a quantity of secretion which varied within the same limits whether the interval between stimulations was one minute, 5, 30, or 60 minutes. My conclusion is that either very little or no secretion at all was produced in the interval, and that the material obtained was secreted reflexly in response to the licking and swallowing movements alone.

Using these 8 dogs, I have also determined the approximate amounts secreted in response to weighed quantities of various materials placed in the mouth and collected through the esophageal fistula together with the secretions produced. It appears that hydrochloric acid is the most potent of the stimuli used, followed in diminishing effectiveness by magnesium sulphate, sodium chloride, and sucrose. Of the foods administered, raw meat induces relatively little secretion; dry meat, much more; and peptone powder, the most of the three. Dry bread crumbs are a much more efficient stimulus than moist bread. In their responses, therefore, the small oral and pharyngeal glands closely resemble the salivary glands.

7292 C

Iodobismitol in Experimental Syphilis.

P. J. HANZLIK.

From the Department of Pharmacology, Stanford University School of Medicine.

In a previous report,[1] it was shown that iodobismitol (N.N.R.) exerts prophylactic and curative effects on the experimental syphilis of rabbits. Since then, confirmations of the antisyphilitic action in rabbits have been made by Levaditi, Vaisman, Manin and Schoen[2] and by Strandberg and Sjögren,[3] da Nova Gomes and de Oleveira,[4] and Carrillo, Schujman and Campos[5] in clinical syphilis, the latter authors agreeing with Mehrtens and Pouppirt.[6] These reports also contain confirmation of other claims for iodobismitol, namely, electronegativity of iodobismuthite, rapid absorption, sustained excretion, low toxicity and positive cerebrospinal penetration. The chemotherapeutic tests begun in this laboratory 7 years ago have been continued and this report submits the final results on the curative effects of iodobismitol together with some interesting comparisons.

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[1] Johnson, Hanzlik, Marshall, and Mehrtens, *J. Pharm. Exp. Therap.*, 1932, **45**, 469.

[2] Levaditi, Vaisman, Manin, and Schoen, *Bull. de la Soc. Franc. de Dermat. et de Syphil.*, 1933, No. 5, p. 738.

[3] Strandberg and Sjögren, *Acta Dermato-Venereologica*, 1933, **14**, 1; Strandberg, *Nord. Med. Tidsk.*, 1932, **4**, 834.

[4] da Nova Gomes and de Oleveira (personal communication).

[5] Carrillo, Schujman, and Campos, *La Semana Méd.*, 1933, No. 48, p. 1.

[6] Mehrtens and Pouppirt, *Arch. Neurol. and Psychiatry*, 1931, **26**, 1220.

The procedure was the same as that used previously,[1] except that during the past 2 years curative treatment with iodobismitol was given in 3 divided doses within 1 week, instead of 1 single dose. Since 1 week of a rabbit's life (expectancy about 8 years) is equal to about 8 weeks of a man's life (expectancy about 60 years), the treatment-period was the equivalent of clinical medication for 2 months, which is a fairly common initial period in practice. The iodobismitol was injected intramuscularly in a range of doses to permit determination of the minimum effective dose and "therapeutic index", if possible. The virus was a strain of *treponema pallida* from a penile chancre, and, by this time, has had 37 passages through rabbits. Fully developed scrotal chancres containing treponema appeared in 3 to 4 weeks after inoculation of the virus. Lymph-node transfers were made 3 months after injection of the bismuth, using generally 3 rabbits, sometimes 4, for tests of lymph-node infectiousness. Virus infected but untreated rabbits were used as controls throughout. Examinations of all rabbits were made routinely in the usual manner. A total of 60 rabbits was used, at least 3 for each dose of iodobismitol. Inasmuch as the results with single and divided doses of the iodobismitol were the same, they were put together, and medians used for the curves in Fig. 1.

The curves in Fig. 1 indicate certain definite tendencies. Doses of less than 20 mg. Na_2BiI_5 (6.5 mg. Bi) per kg. were ineffective, since the duration-periods for chancres and treponemata were grouped around those for the untreated rabbits. There was some evidence of antisyphilitic action with 30 mg. Na_2BiI_5 (6.5 mg. Bi) per kg., but not of curative action since the lymph nodes remained unsterilized (positive). More definite antisyphilitic and curative actions occurred with doses of Na_2BiI_5 from 40 to 120 mg. (8.6 to 25.8 mg. Bi) per kg. The minimum curative dose, therefore, might be around 35 mg. Na_2BiI_5 per kg., although this was not actually tested. It seemed clear that rate of clearing and healing of chancres increased with increasing dosage of bismuth. The periods for treponema and chancre were 3 and 6 weeks, respectively, with the minimum effective dose, and about 1 and 5 weeks, respectively, with

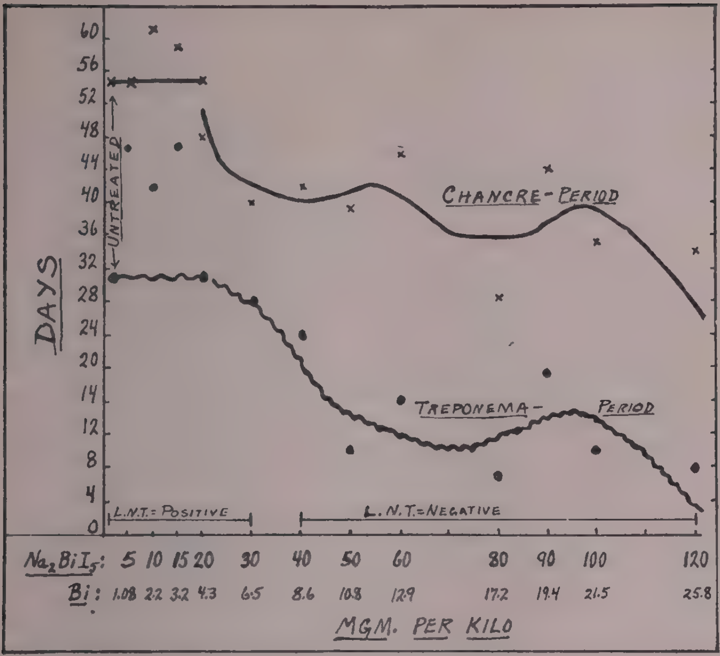

FIG. 1.

Curative Effects of Iodobismitol in Experimental Syphilis of Rabbits. Duration of chancres and *treponema pallida* are indicated by the curves. Iodobismitol (N.N.R.) contains: sodium iodobismuthite (Na_2BiI_5) 6% and sodium iodide, 12%, in ethylene glycol. L. N. T. = lymph node transfer.

the maximum dose used. Presumably, therefore, efficiency of the bismuth treatment was related to concentration of bismuth in blood and tissues. Such relationship, however, would indicate nothing of the fundamental nature of the chemotherapeutic mechanism.

The therapeutic index can be only approximately indicated, since toxicity tests[7] of iodobismitol were made with single injections and curative tests chiefly with divided injections. The only justification for the comparison is that the results of curative tests with single and divided doses were similar. Since 120 mg. Na_2BiI_5 (in ethylene glycol) per kg. was the M.F.D. (70% mortality) for rabbits, and 40 mg. was the minimum curative dose, the therapeutic index was 3. The M.F.D. of iodobismitol prepared with propylene glycol was over 200 mg. Na_2BiI_5 per kg.,[8] therefore the index might be over 5. For an antisyphilitic action, without complete sterilization of lymph nodes, such as after 30 mg. Na_2BiI_5 per kg., the index might be from 4 to over 6. On the whole, the therapeutic index of iodobismitol compared favorably with that of other bismuth products.

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[7] Hanzlik, Seidenfeld, and Johnson, *J. Pharm. Exp. Therap.*, 1932, **46**, 1.

[8] Hanzlik, Mehrtens, and Spaulding, *J. Pharm. Exp. Therap.*, 1933, **49**, 300.

If the results on rabbit syphilis be compared with those on clinical syphilis[6] according to dosage per kilo body weight and period of medication in relation to life expectancy, and similar antisyphilitic actions be assumed for bismuth, in the 2 species, an interesting agreement is observed. Using 40 mg. Na_2BiI_5 per kg. as the minimum curative dose for experimental syphilis, and assuming 1 week of a rabbit's life is equal to about 8 weeks of a man's life, then for a 70 kilo adult the estimated total dose of iodobismuthite for definite antisyphilitic effects is 2.8 gm., or 0.35 gm. weekly in 3 divided doses of 0.12 gm. for 2 months. Actually, Mehrtens and Pouppirt[6] found that courses of medication consisting of 2 to 3 such doses of 0.12 gm., or a total of from 0.24 to 0.36 gm. weekly, for 2 to 3 months produced beneficial effects on advanced clinical syphilis in their report on 200 patients. This general agreement is about as good as might be expected, and probably not accidental, although comparisons with acute clinical syphilis might give different values. According to the criteria used, these chemotherapeutic tests on experimental syphilis would appear to be satisfactory for assessing the possible clinical antisyphilitic value of bismuth products.

Conclusions. Iodobismitol is definitely curative in experimental syphilis of rabbits. The minimum curative dose appears to to be about 40 mg. Na_2BiI_5 (8.6 mg. Bi) per kilo body weight, which agrees with clinical tests according to certain criteria. The therapeutic index is about 3, or possibly 4 or over 6. Confirmations by others of antisyphilitic actions of iodobismitol in experimental and clinical syphilis are reported.

7293 P

New Formulae for Prediction of Basal Metabolism from Pulse Rate and Pulse Pressure.

J. MARION READ AND CHARLES W. BARNETT.

From the Department of Medicine, Stanford University School of Medicine.

In a previous study[1] one of us derived a multiple prediction formula for estimating basal metabolic rate from pulse rate and pulse pressure. Recent interest in the clinical usefulness of this formula made it advisable to reinvestigate this problem and attempt to increase its reliability if it is to be applied clinically. Pulse rate and pulse pressure changes which were found to parallel variations in basal metabolic rate were interpreted as indicative of accompanying alterations in blood flow. The subsequent development of methods for measuring blood flow, and their applications in myxedema and thyrotoxicosis, have demonstrated that heat production, and minute volume closely parallel each other. Simultaneous determinations of blood pressure show that minute volume can be roughly estimated clinically by determining the pulse rate and blood pressure. We first assumed that minute volume varied directly with the product of the pulse rate and the pulse pressure. However, resting minute volume must also vary with the individual's size, so this factor was introduced by multiplying the pulse rate-pulse pressure product by surface area. Combining the above equations, we obtain:

$$\text{Total heat production} = K \cdot \text{Pulse Pressure} \cdot \text{Pulse Rate} \cdot \text{Surface Area.}$$

Dividing both sides of the equation by surface area gives:

$$\text{Calories per sq. m. per hour} = K \cdot \text{Pulse Rate} \cdot \text{Pulse Pressure.}$$

The next step was to ascertain whether or not observations on patients would fit this equation. When the data were assembled, it became evident that the products of pulse rate and pulse pressure were in general higher, and the heat production lower, in women than in men. The derivation of separate formulae for the 2 sexes was therefore essential. Observations were recorded on 416 women and 321 men. A correlation table was prepared for each sex and the equations for the regression lines were determined in the usual way. The simplified final equations are:

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[1] Read, J. Marion, *Arch. Int. Med.*, 1924, **34**, 553.

Men

$$\text{Cal. per sq. m. per hr.} = \frac{\text{P.R.} \times \text{P.P.}}{200} + 27$$

Women

$$\text{Cal. per sq. m. per hr.} = \frac{3 \times \text{P.R.} \times \text{P.P.}}{700} + 24$$

The correlation coefficients were slightly over 0.8 in each case.

Since the basal metabolic rate is expressed as a percentage above or below fixed normal standards for age and sex, it is evident that any attempt to derive a formula giving the B.M.R. directly must increase the scatter of the observations. We therefore determine only heat output from which the B.M.R. must be calculated in the usual way. This new procedure, together with the separation of the sexes and introduction into the formula of the individual's size have all operated to increase the accuracy of our prediction formulae.

To ascertain their prediction value the formulae were applied to 100 consecutive new cases, omitting from the series all cases with arrythmias, hypertension or other cardiovascular disease which produced alterations in the pulse rate and pulse pressure obviously not caused by changes in blood flow. These were the same criteria used in compiling the data from which the formulae were derived. There were 21 males and 79 females in this new series to which the formulae were applied with the following results:

ERROR NOT MORE THAN

5%	10%	15%	20%	25%
59	78	94	98	100

Of the 22 cases in which the disagreement between the measured and predicted rate exceeded 10%, nine of the paired values both fell within the accepted normal range of —10% to +10%. Of the remaining 13, two gave normal and 11 abnormal rates by indirect calorimetry. Of these 11 the direction of metabolic change was the same in both pairs in 10, and in the 11th the values were +1% and —12%.

In judging the value of any formula for estimating the basal metabolic rate it should be recognized that the standard to which we compare the estimated rate is not determined with perfect accuracy. The variable error inherent in the complexity of indirect calorimetry has been discussed by Jenkins[2] and others. In fact we have made additional use of the pulse rate-pulse pressure prediction formula to detect gross errors in metabolic rate determinations by indirect calorimetry. The pulse rate alone has proven very useful for many years for this same purpose.

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[2] Jenkins, R. L., *Arch. Int. Med.*, 1932, **49**, 181.

It is probable that the accuracy and clinical value of our formulae may be enhanced by altering its constants, which may be advisable after recalculation upon a larger series.

7294 P

Bacteriology of Leprosy. II. Growth and Staining Reactions of Organisms Inoculated into Minced Chick Embryo Medium.

A. J. SALLE and J. R. MOSER.

From the Department of Bacteriology, University of California, Berkeley, California.

In a previous communication[1] a description was given of an acid-fast organism isolated from human and rat leprous lesions by the tissue culture technic.

Some of the organisms were acid-fast and some non-acid-fast in fresh tissue cultures and in minced embryo medium, but entirely acid-sensitive on the usual laboratory media.

The best growth of acid-fast organisms occurred in from 24-48 hours. There were usually as many acid-fast as acid-sensitive organisms present. After this period of incubation the acid-fast rods gradually disappeared and on about the tenth day the organisms were non-acid-fast or almost so. On transferring to fresh tissue cultures or minced embryo medium, the above picture was repeated.

The tinctorial characteristics varied depending upon the living condition of the tissues. In vigorous, actively growing tissues the organisms were strongly acid-fast. As the tissues became less vigorous the acid-fast property was less pronounced and, finally as the tissues died and autolyzed, only non-acid-fast diphtheroids were seen.

The organisms isolated from human and rat lesions revealed the same morphological and physiological characteristics. It was, therefore, concluded that human and rat leprosy are caused by the same organism.

In order to determine whether or not the acid-fast phase is characteristic of bacteria in general, when inoculated into minced embryo medium, the following organisms were tested: *Escherichia coli*, *Eberthella typhi*, *Eberthella dysenteriae*, *Eberthella paradysen-*

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[1] Salle, A. J., *J. Infect. Dis.*, in press.

teriae (Bacillus dysenteriae Flexner), *Bacillus subtilis, Bacillus subtilis* (dissociating strain), *Bacillus anthracis, Corynebacterium diphtheriae* (American No. 8), *Corynebacterium xerosis, Corynebacterium pseudodiphthericum (hoffmanni), Staphylococcus aureus, Sarcina lutea, Rhodococcus roseus, Clostridium botulinum* (Type A), *Clostridium welchii* (Type 1), *Clostridium tetani, Streptococcus pyogenes* (beta or hemolytic strep.), *Streptococcus mitior* (alpha or viridans strep.), *Hemophilus influenzae, Neisseria intracellularis* (meningococcus), *Diplococcus pneumoniae, Saccharomyces cerevisiae* (baker's yeast), *Sporotrichum schencki, Monilia krusei, Epidermophyton cruris, Acladium castellani, Blastomyces hominis, Actinomyces violaceum, Actinomyces* isolated from air.

The minced chick embryo medium was prepared in the following manner:

Minced chick embryos (9-12 days)	1 part
Tyrode solution	5 parts
	6 parts

The embryos were decapitated, after which they were minced in a tissue grinder and mixed with Tyrode solution in the above proportion. The heads were removed[2] because the pigment granules contained in the eyes often led to confusion when smears were made to determine the sterility of the medium or to note presence or absence of growth of the inoculated organisms. About 3 cc. amounts of the medium were measured into test tubes, after which it was ready for use.

Smears were stained for 30 minutes in cold carbol-fuchsin, decolorized with 10% sulfuric acid and then counterstained with Loeffler's methylene blue.

The results showed that, with the exception of the yeast *Saccharomyces cerevisiae* and the mold-like organism *Actinomyces violaceus,* none of the organisms retained the fuchsin stain when treated with 10% sulfuric acid. The true bacteria were acid sensitive when stained by the acid-fast technic. Therefore, it may be stated that the ability of an organism to retain the acid-fast stain in minced chick embryo medium is not a phenomenon characteristic of the true bacteria (Eubacteriales), but restricted so far to organisms falling into other orders (Actinomycetales, etc.).

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[2] Rivers, T. M., *J. Exp. Med.*, 1931, **54**, 453.

7295 P

Bacteriology of Leprosy. III. Growth and Staining Reactions of Acid Fast Organism Inoculated into Minced Animal Tissues.

A. J. SALLE AND J. R. MOSER.

From the Department of Bacteriology, University of California, Berkeley, California.

An acid-fast organism isolated from human and rat leprous lesions in tissue cultures, has been described.[1] It is believed that this organism represents the true etiological agent of leprosy because the same organism has been isolated from several human nodules and from rat granuloma.

Acid-fast organisms were present only when minced chick embryo was used as the culture medium. On ordinary lifeless media the organisms failed to retain the fuchsin dye, but took the counterstain.

It was also found[2] that the ability of an organism to retain the acid-fast stain in minced chick embryo medium is not a phenomenon characteristic of the true bacteria (Eubacteriales), but restricted so far to organisms classified with other orders (Actinomycetales, etc.).

The question that naturally arose was whether or not the organisms became acid-fast when inoculated into minced tissues obtained from laboratory animals. Organs from full grown rabbits, guinea pigs and rats were used. The tissues were removed aseptically and minced separately in tissue grinders. The minced tissues were mixed with Tyrode solution in the following proportion: Minced tissue, 1 part; Tyrode solution, 5 parts. The media were pipetted into test tubes, about 3 cc. to each tube, after which they were ready for use.

Growth occurred within 48 hours in all of the cultures with the exception of those made with rat tissues. The results from rat tissues were much poorer than from media prepared by mincing rabbit and guinea pig tissues. Only very few acid-fast organisms were observed, and the growth was very poor.

The best results were obtained from guinea pig tissues. The number of acid-fast organisms present was even greater than when minced chick embryo medium was employed.

In the rabbit medium the pigment production appeared to be enhanced. With the other tissues pigment production was normal.

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[1] Salle, A. J., *J. Infect. Dis.* (in press).

[2] Salle, A. J., and Moser, J. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 725.

The organisms lost their acid fastness after about 5 days of incubation, reverting again to the acid-sensitive forms.

The results show that living, embryonic tissue was not necessary for the appearance of the acid-fast forms; dead sterile minced tissues obtained from adult rabbits, guinea pigs and rats produced results equally as good.

7296 C

Olfactory Tract and Poliomyelitis.*

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From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

Faber[1] has recently reviewed clinical and experimental evidence in support of the view that acute poliomyelitis is primarily a disease of the central nervous system. It is well established that virus deposited on the nasal mucous membranes in some way gains admission to the central nervous system. We have described[2] a method which has enabled us to infect 95% of monkeys inoculated with virus by the intranasal route. While it may be assumed from the evidence already reported that the virus gains entrance to the central nervous system by the way of the olfactory nerve no satisfactory proof that this is the normal route has thus far been produced. The observations we desire to report seem to offer this final evidence. This rests on a failure to infect monkeys by the intranasal route after the olfactory tracts have been sectioned with an electric cautery.

Six *Macacus rhesus* monkeys were subjected (12/6/33) to the following operation while under deep anesthesia: An incision approximately 1.5 cm. in length was made through the skin and subcutaneous tissues in the mid-nasal line almost immediately above the level of the supraorbital ridges. With a Stille's bone drill, provided with a suitable guard, a round opening, about 0.8 cm. in diameter was drilled into the frontal bone. This opening was made at approximately the level of the olfactory bulbs. With a specially

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* These studies were supported by the Mrs. Mary Hooper Somers Medical Research Fund.

[1] Faber, H. K., *Medicine*, 1933, **12**, 83.

[2] Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1010.

made electric cautery tip (0.4 cm. x 5.5 cm.), the region of the olfactory bulb and olfactory tract for a distance of about 2 cm. from the opening, was subjected to cauterization. After suturing the skin and covering the area with collodion the animals were held for a half hour in a specially designed box animal-holder to prevent them from injuring the operative field while recovering from the effects of the anesthetic. None of the animals seemed disturbed by the operation and the wounds in each instance healed nicely.

Fifteen days after cauterization all of the monkeys, together with 3 normal controls, were given intranasal instillation of virus according to the procedure already described by us.[2] Briefly stated this procedure consists of 3 intranasal instillations (0.5 cc. each) of virus (10% M V suspension), administered on the same day, each instillation being preceded by a thorough intranasal irrigation with a M/15 phosphate buffer solution, pH 5.†

The results of the experiment were striking. Out of a total of 9 monkeys inoculated with virus only the control monkeys developed the disease (all of these on the 7th day). Sixteen days following the first inoculation the 6 operated monkeys were again subjected to 3 intranasal instillations of virus according to the procedure described. This time 2 controls were employed. These developed poliomyelitis 7 days later, while the operated animals continued to remain well. Three weeks following the second intranasal instillation of virus all of the operated monkeys were inoculated *intracerebrally* with poliomyelitis virus (5% Berkefeld V filtrate) to test their susceptibility to virus administered by the intracranial route. All developed typical poliomyelitis 5 to 8 days later.

Each animal was exsanguinated under deep ether anesthesia and the brain and cord were removed. In removing the brain particular attention was given to the nervous tissue in the region of the olfactory bulb and tracts to determine whether or not the olfactory tracts had actually been severed by the cauterization. Complete severance of *both tracts* was verified in each instance. The portion destroyed generally extended from the region of the bulbs to about 0.5 cm. rostral to the point where the individual tracts divide to form the medial and lateral stria. The uncauterized remains of the olfactory tracts were seen as "stubs" with free, slightly curled, ends where the cautery had severed the tracts.

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† To properly carry out the preliminary intranasal irrigation and also the intranasal instillations of virus it is necessary to put the monkey under deep anesthesia. To irrigate the nasal passages we put the monkey in a special box-holder with chin down and body well elevated. The irrigation is carried out with a large (30 cc.) Luer syringe, provided with a short (6 inches) rubber tube ending in a bulb which fits snugly into the external nares. A highly active virus suspension is necessary.

The results of this experiment strongly support the view that the virus normally passes from nasal mucous membranes to the central nervous system by the way of the olfactory nerve, olfactory bulb, and olfactory tracts. Only in so far as the cauterization may have destroyed possible vascular communications does the evidence lack finality.

7297 C

A Simplified Method for Measurement of Creatinine Clearance.

R. F. HANZAL AND J. M. HAYMAN, JR.

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The multitude of substances whose "clearances", or rate of excretion in relation to blood concentration, have been proposed as tests of kidney function indicates that a satisfactory clinical procedure is yet to be devised. The use of urea, as proposed by Möller, McIntosh and Van Slyke,[1] is in our experience the simplest of the more sensitive tests, since it involves only one blood sample and does not require the ingestion of the test substance. But since the rate of urea excretion is less with low urine volume than with high, function tests can be compared only as a percentage of an average empirical normal for urine volumes above or below 2 cc. per minute. There are, therefore, certain advantages in using a test substance whose excretion is independent of urine volume. Creatinine, the use of which was proposed by Rehberg,[2] is such a substance. This method as described[3] has the disadvantage of requiring ingestion of creatinine, and analysis of urine and 2 samples of blood plasma.

The development by Van Slyke and Cope[4] of a clinical method for the determination of urea clearances suggested that a similar procedure might be devised for the creatinine clearance which, if practical, would considerably simplify the test.

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[1] Möller, E., McIntosh, J. F., and Van Slyke, D. D., *J. Clin. Invest.*, 1928, **6**, 427.

[2] Rehberg, P. B., *Biochem. J.*, 1926, **20**, 447.

[3] Rehberg, P. B., *Zentr. f. Inner. Med.*, 1929, **50**, 367.

[4] Van Slyke, D. D., and Cope, C. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1169.

In the method of Van Slyke and Cope, the ratio between urine and blood urea concentrations is determined by a direct comparison, without determining the actual concentration in either. In the method here described, the same principle is applied except that creatinine is used. In order to increase the concentration of creatinine in the blood and urine, creatinine is administered to the subject by mouth, and water given at hourly intervals during the test if there is any question of the subject's ability to urinate.

The amount of creatinine ingested depends upon the number of hourly urine collections to be made. For a single collection 3 gm. were found sufficient; for 2 periods 5 gm. were better, but if 6 or 7 collections are to be made as much as 10 gm. will be necessary. Following the ingestion of 3 gm. of creatinine on an empty stomach, the blood concentration reaches a maximum in 1 hour, but with larger amounts the highest concentration is not attained until about 1½ hours. At the end of the 1 or 1½ hours, the bladder is emptied and urine discarded. Urine is then collected as accurately as possible for a measured interval of time, approximately 1 hour. The actual time is recorded to the nearest minute. A sample of venous blood is drawn at the middle of each period. Coagulation is prevented by the use of potassium oxalate.

Only 2 reagents are needed in this method: solid picric acid which has been purified to meet the requirements of Folin and Doisy,[5] and a 10% solution of sodium hydroxide. The urine is diluted (in round numbers) as many times as is indicated by the curve of Figure 1, which has been constructed similarly to that of Van Slyke and Cope.[4] Two cubic centimeters of plasma are added to 18 cc. of water in a 50 cc. centrifuge tube and mixed. Twenty to 25 cc. of the diluted urine are placed in a similar centrifuge tube and approximately a gram of picric acid added to both. The mixture is thoroughly stirred with a glass rod until it is uniformly yellow and then allowed to stand with occasional stirring for 20 to 30 minutes after which it is centrifuged and filtered. It is essential that a clear filtrate be obtained.* Ten cc. portions of the picric acid filtrates are placed in test tubes and 0.5 cc. of the 10% sodium hydroxide added to each. The test solutions are then mixed and allowed to stand for 10 minutes before comparison is made in a colorimeter. The cup containing the blood test solution is set at

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[5] Folin, O., and Doisy, F. A., *J. Biol. Chem.*, 1917, **28**, 349.

* Schleicher and Schüll filter paper number 597 has been found satisfactory.

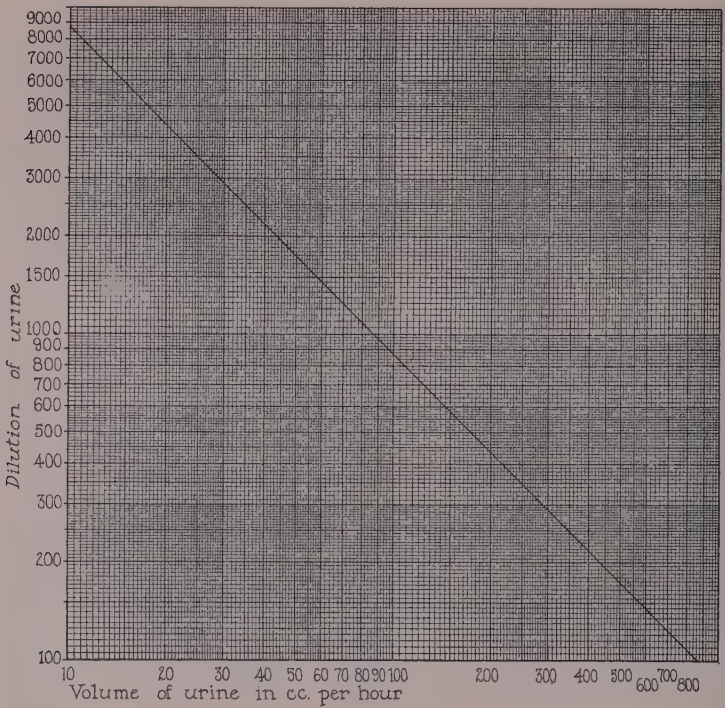

FIG. 1.

20.0 mm. and the cup containing the urine test solution is adjusted so that the two match.

Calculation. The equation for calculation of results is as follows:

$\frac{Rb}{Ru} \times \frac{Du}{Db} \times V =$ creatinine clearance in cc. per minute where Rb and Ru represent the colorimeter readings of the blood and urine test solutions respectively, Du and Db are the dilutions made of the urine and blood, and V is the number of cc. of urine excreted per minute. To express the clearance in percent of the average normal value the equation becomes:

$\frac{Rb}{Ru} \times \frac{Du \times V \times 100}{Db \times 148} = \%$ of average normal creatinine clearance, when 148 cc. is taken as the average normal for adults.[6]

Table I shows the results obtained by 2 observers working independently. One used Rehberg's modification of Folin's method,

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[6] Hayman, J. M., Jr., Halsted, J. A., and Seyler, L. E., *J. Clin. Invest.*, 1933, **12**, 861.

TABLE I.
Comparison of Results Obtained in Present Method and Rehberg Method.

Subject No.	Observed Clearance This Method cc.	Observed Clearance Rehberg cc.	% Average Normal Clearance This Method	% Average Normal Clearance Rehberg	Difference in % Average Normal
1	128.9	125.0	87.1	84.5	+ 2.6
2	132.6	123.5	89.5	83.4	+ 6.1
	167.9	185.0	113.4	125.0	—11.6
	161.7	162.5	109.3	109.7	— 0.4
	163.1	167.0	110.2	113.6	— 3.4
3	103.6	107.7	69.9	72.7	— 2.8
	91.0	101.8	61.5	68.8	— 7.3
	81.7	85.4	55.1	57.7	— 2.6
	85.7	83.0	57.8	56.0	+ 1.8
	96.7	84.0	65.3	56.7	+ 8.6
	109.4	96.1	74.0	65.0	+ 9.0
4	35.1	33.8	23.7	22.8	+ 0.9
	41.1	45.0	27.8	30.4	— 2.6
5	143.1	153.0	96.8	103.4	— 6.6
	114.4	115.0	77.3	77.7	— 0.4
6	106.0	103.0	71.6	69.6	+ 2.0
	93.1	99.5	62.9	67.2	— 4.3
	90.1	96.5	60.9	65.2	— 4.3
	92.9	93.5	62.7	63.1	— 0.4
	101.7	96.5	68.9	65.2	+ 3.3
	87.8	86.5	59.3	58.4	+ 0.9
	98.5	101.0	66.5	68.2	— 1.7
7	10.5	11.6	7.1	7.8	— 0.6
	10.9	11.5	7.3	7.8	— 0.5
8	20.9	18.4	14.1	12.4	+ 1.7
9	138.5	136.5	93.5	92.2	+ 1.3
10	51.4	49.5	34.7	33.4	+ 1.3

employing a colorimeter with Burker optical system, and the other the present method.

Summary. A simplified method of obtaining the ratio of concentration of creatinine in urine and blood plasma for calculation of the creatinine clearance is described.

7298 C

Effect of Chlorides upon Distribution Ratio of Bromides in Blood and Cerebrospinal Fluid.

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It was found[1] that the quantitative determination of bromides by the Walter method was influenced by the variations in the chloride concentration. In the presence of chlorides the bromides cannot be completely recovered by this test, and the loss is directly proportional to the degree of concentration of chlorides. It was pointed out that the use of distilled water in diluting the blood serum to be examined for bromides, introduced an error in that the loss of bromides in the blood was proportionally less than the loss in the cerebrospinal fluid. For this reason it was suggested that the blood should be diluted with a NaCl solution which will raise the concentration of chlorides in the serum to the degree found in the cerebrospinal fluid. Seeing that most of the reports on the distribution of bromides were made on the basis of investigations in which the blood was diluted with distilled water, we here report a series of cases in which this error was corrected.

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[1] Malamud, W., Mullins, B. M., and Brown, J. R., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1084.

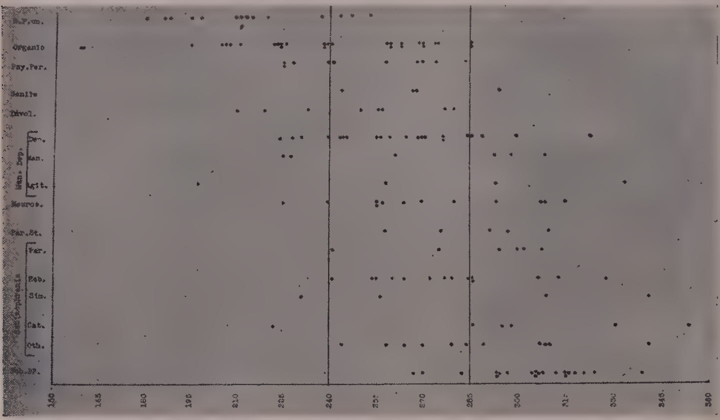

FIG. 1. Ratio of distribution of bromides in mental diseases.
Figures at the bottom refer to the permeability quotient (blood bromides: cerebrospinal fluid bromides). Designations along left-hand margin refer to different disease syndromes, *viz.:* general paresis, untreated; organic brain disease; psychopathic personality; senile psychoses; involutional psychoses; 3 subgroups of manic-depressive psychosis; neuroses; paranoid states; 5 subgroups of schizophrenia and schizoid psychopathies.

In Fig. 1 we have arranged the findings in 173 cases representing different forms of mental and nervous diseases on the basis of the distribution ratio of the bromides. The first thing that becomes apparent is that these ratios fall distinctly below those that have been reported previously.[2] The highest quotient in this chart is 354, whereas the low ones reach the level of 158. With this we also find that diseases such as psychopathic personalities, senile psychoses, etc., which have previously been shown to have a normal ratio varying between 280 to 320 in the present chart have fallen to a level predominantly ranging between 240 and 285. In other words, the absolute figures in determinations, where the error introduced by the distilled water is corrected, fall distinctly below the figures where this was not taken into consideration. Analysis of the chart shows, however, that the relative proportions of these figures and their relationships to different types of diseases remain practically the same as in previous publications. Thus we find that the untreated general paretics show consistently low figures; that is, the penetration of bromides into the cerebrospinal fluid is increased. This is also true but not quite to the same extent of the organic psychoses. On the other hand, the schizophrenias, schizoid psychopathies, and the paranoid states here too show a large proportion of increased figures (decreased permeability). A smaller portion falls within the new established normal limits, and only 2 of the cases are below the normal level. Similarly the manic depressive psychoses, the involutional mental diseases, and the neuroses show the same relative proportions as in previous investigations. This would justify the conclusion that although the true ratios in all of these cases are different from those obtained before, the characteristic deviations from normal and the interrelationships between the different types of diseases remain very much the same as before.

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[2] Malamud, W., Fuchs, D. M., and Malamud, N., *Arch. Neurol. and Psychiat.*, 1928, **20**, 780.

The chloride contents of the blood and cerebrospinal fluid in most of the diseases reported in the above chart do not differ very much from the normal. In the normal person the average blood chloride content is about 600 mg. per 100 cc., and that of the spinal fluid is about 720 mg. This ratio seems to hold true for a large proportion of mental diseases, varying between narrow limits. It was therefore felt that in most cases if the blood is diluted with a NaCl solution of a somewhat higher concentration than that of the spinal fluid, the resulting loss of bromides will be equal in both fluids and, therefore, will not influence the bromide ratio obtained. In some cases, however, especially of organic diseases, the chloride content either of the blood or of the cerebrospinal fluid is changed in a pronounced fashion, and in such conditions it is necessary to correct the findings before the ratio is obtained. For that purpose it was felt necessary to determine the exact amount of loss of bromides in different concentrations of NaCl and on this basis establish a set of figures that could be utilized in the determination of the bromide ratio in such cases. We have tabulated in a tri-dimensional graph the relationships between the amount of bromides obtained to that actually present in solutions of NaCl of different concentrations. This graph to be used for purposes of correction in this method.

Known quantities of sodium bromide in the proportion of 20, 30, 40, and 50 mg. per 100 cc. were added to solutions of NaCl in water in concentrations of 400, 500, 600, 700, 800, and 900 mg. per 100 cc. The quantities obtained in each case were determined, and on that basis the above mentioned graph was prepared. With the aid of the above mentioned graph, given a certain figure representing mg. of bromide per 100 cc. and knowing the quantity of chlorides in that fluid, the amount of bromides actually present in the fluid can be computed. The graph was prepared on the basis of solutions of 20, 30, 40, and 50 mg., and as the loss seems to increase fairly regularly with the increase in chloride concentration we felt justified in assuming the figures between these quantities by dividing them into 10 parts. The error introduced up to about 50 mg. of chlorides in 100 cc. is so small that it was felt that in cases where the chlorides do not deviate from the normal in a very pronounced fashion the correction is not necessary, as the margin of error is not greater than the error usually introduced by colorimetric determinations.

7299 P

Skin Temperature Changes After Total Thyroidectomy.

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As a part of the careful study of patients submitted to the operation of total thyroidectomy for heart disease, we have conducted skin temperature observations before and after the operative procedure. We have utilized the method of Gibbon and Landis.[1] This method has been shown to produce adequate vasomotor dilatation and is far simpler than the other methods of injection of foreign proteins, the induction of a general or spinal anesthetic, or the blocking of sympathetic ganglia or peripheral nerves with novocaine or alcohol.

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[1] Gibbon, J. H., Jr., and Landis, E. M., *J. Clin. Invest.*, 1932, **11**, 1019. Landis, E. M., and Gibbon, J. H., Jr., *Arch. Int. Med.*, 1933, **52**, 785.

The operative procedure on all patients was done under local anesthesia, thereby eliminating the variability of blood flow studies as seen after a general anesthesia (Herrick *et al.*[2]). The skin temperature determinations were all done in a small room, free from air currents, and having a constant temperature of 68-72°F. and humidity between 40 and 50%. This gives constant findings as shown by Talbot.[3] The patients in all cases were in bed in a semi-Fowler position and the points of election of skin temperature were the tibial tubercle, anterior ankle, and great and small toes of both lower extremities.

According to Morton and Scott,[4] there is a maximum vasodilatation response for normal vessels that has been designated the "normal vasodilatation level". The lower limit of this maximum level for the surface temperature of the great toe whose vasoconstrictors have been released by general or spinal anesthesia (and the water-bath technique of Gibbon[1]) is 31.5°C. at a room temperature of 20 C. (69 F.).

Studies of the skin temperature were made on 13 patients before

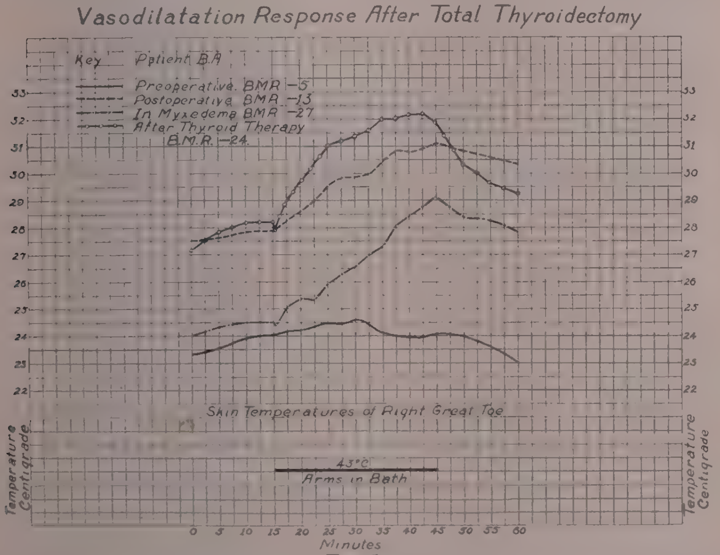

FIG. 1.

Vasodilator ability in relation to thyroid function.

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[2] Herrick, J. F., Essex, Hiram E., and Baldes, Edward J., *Am. J. Physiol.*, 1932, **101**, 213.

[3] Talbot, Fritz, *Am. J. Dis. Child.*, 1931, **42**, 965.

[4] Morton, John J., and Scott, W. J. Merle, *New Eng. J. Med.*, 1931, **204**, 955.

operation and the vasodilator response determined. In these same patients postoperative studies were carried out at varying intervals, as from the fourth postoperative day to about 7 weeks later when the patient was in clinical myxedema, and again after thyroid therapy. The results of these studies in a typical case of angina pectoris are shown in Fig. 1. These results indicate what was anticipated, a definite link between the thyroid and the sympathetic vasomotor apparatus. That the adrenals play an important rôle in this mechanism is shown in Fig. 2. A patient with cardiac valvular disease (compensated) before operation was given 300 cc. of ice water at 0-1°C., to drink rapidly. This causes an outpouring of adrenalin into the circulation as has been shown by Cannon *et al.*[5] The response was characterized by a rise in blood pressure and pulse rate; the water-bath skin temperature test showed a normal response. The complete test was repeated 4 days after operation. There was then only a slight rise in blood pressure and pulse rate with a higher initial skin temperature. This definitely suggests a diminished sympatheticotonic-adrenal activity following total thyroidectomy.

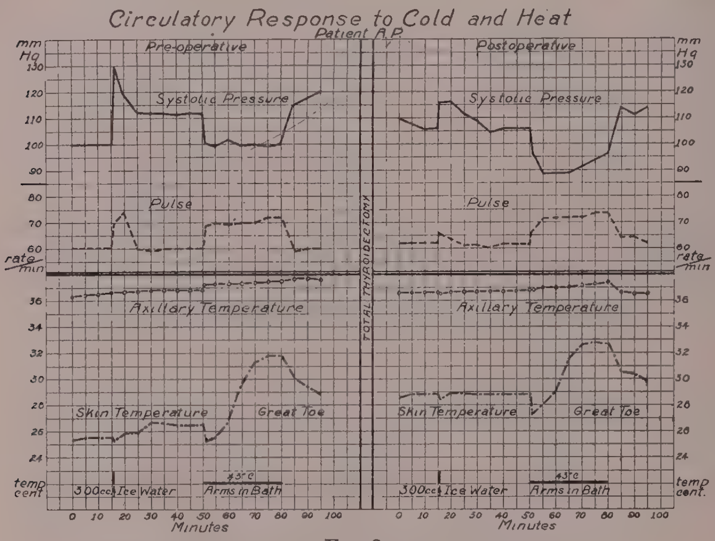

FIG. 2.
Sympathetic-adrenal activity in relation to thyroid function.

It would appear that the vasodilatation response in patients whose thyroid gland has been removed *in toto* might suggest some utilization of this finding in the future treatment of patients with peripheral vascular disease. At least it appears that the increased dilatation response is as great as that achieved by operations upon the sympathetic apparatus, whether central in type, that is, ganglionectomy or ramisection, or peripheral in type, that is, decortication of the arteries.

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[5] Cannon, W. B., Querido, A., Britton, S. W., and Bright, E. M., *Am. J. Physiol.*, 1927, **79**, 466.

Moreover, it would appear that, even with observations carried on for only 4 months, we have already indicated as permanent a vasodilatation response as the various sympathetic procedures have given us up to this time.

7300 C

Cobalt Glutamate in Nutritional Anemia.*

ERWIN BRAND AND CHARLES J. STUCKY.

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While our studies on milk anemia[1, 2] were in progress, evidence appeared in the literature[3, 4] which indicated that cobalt stimulated blood regeneration in nutritional anemia. Therefore, experiments were conducted to determine the effect of cobaltous glutamate as compared to inorganic cobalt in hematopoiesis, when supplementing a whole milk (klim)-iron-copper diet. The technique was as previously described.[1]

In preliminary experiments it was found that rats, which had recovered from anemia by supplementing the whole milk (klim) ration with Fe and Cu showed renewed hematopoietic activity when the diet was further supplemented with 0.5 mg. cobalt as cobalt nitrate. Thus at the end of a feeding period of 6 weeks, a group of 3 "cobalt" rats gave average values of 18.6 gm. and 11.0 millions for the hemoglobin and erythrocytes respectively as compared to

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* In coöperation with the Research Department of the Calco Chemical Co., Bound Brook, N. J.

[1] Brand, E., and Stucky, C. J., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 627.

[2] Brand, E., and Stucky, C. J., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 689.

[3] Orten, J. M., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1932, **96**, 11.

[4] Orten, J. M., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1933, **99**, 457.

14.6 gm. and 8.4 millions for a group of "non-cobalt" controls. Both groups gave approximately equal values for these constituents at the beginning of the experiment.

Accordingly, further tests were performed with young anemic animals. Four groups of 3 rats each were employed. The animals were distributed in each group according to sex and litter in order to obtain more representative results. Groups 1 and 3 received 0.5 mg. of ferrous glutamate plus 0.1 mg. of copper glutamate and served as controls for groups 2 and 4 respectively. The cobalt (0.5 mg.) was administered as a bivalent compound of glutamic acid in group 4 and as cobalt nitrate in group 2.

The average results for the groups are shown graphically in Figures 1 and 2 and it can be seen that all the animals showed rapid blood regeneration. However, in the "cobalt" rats, the hematopoietic response was much more extensive, the individual hemoglobin levels reaching values of 18 to 24 gm. and the erythrocytes increasing to 13 to 16 millions. There is no significant difference between the two cobalt groups (*i. e.*, groups 2 and 4) as regards the degree of polycythemia. However, the rats which received cobalt as the inorganic salt (group 2) did not grow as well as the control rats (group 1) and the growth was below that exemplified by the

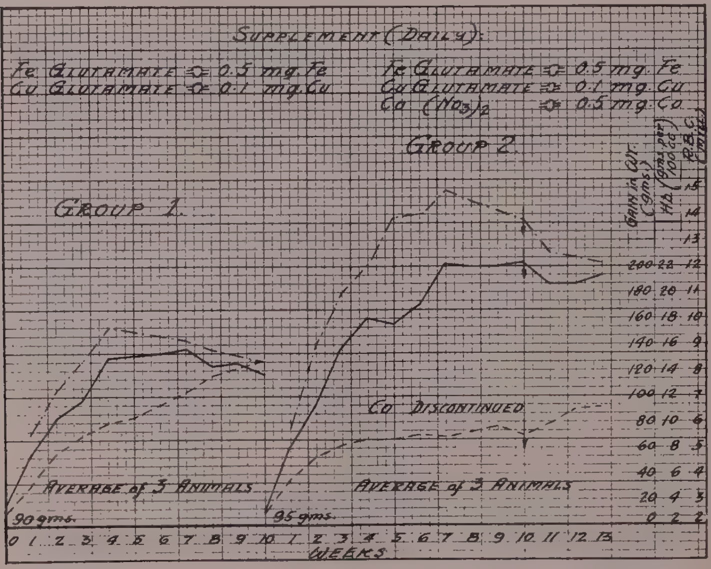

FIG. 1.
Effect of Cobalt Nitrate on Hb, R.B.C. and Growth.

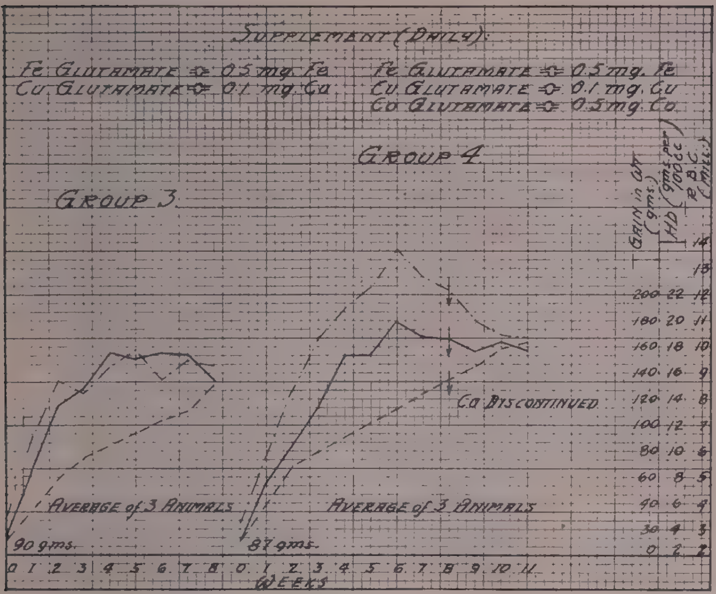

FIG. 2.
Effect of Cobaltous Glutamate on Hb, R.B.C. and Growth.

animals (group 4) which received the cobalt in the form of the glutamate compound. On the other hand, the cobaltous glutamate rats (group 4) grew at the same rate as the controls (group 3). Further experiments should be conducted to determine whether the retardation in growth of the rats which received cobalt in the form of the nitrate salt is significant.

The animals in group 4 gave values of 37% and 47% higher for Hb and erythrocytes respectively than those in group 3. Similarly, the rats in group 2 reached levels of 15% and 41% higher than those in group 1 for these same constituents.

In both cobalt groups there was a gradual decrease in the values for the blood constituents after the cobalt was discontinued. In the case of some of the rats there was a noticeable decrease even before the cobalt supplements were withdrawn. It is possible that this decline in Hb and erythrocytes during the period of cobalt feeding may indicate a toxic effect of this element due to its accumulation in the tissues. Further research may clarify this problem.

From these data it is clear that cobalt accelerates the hematopoietic activity of Fe and Cu in nutritional anemia. A marked polycythemia is produced. No significant difference in this respect could be demonstrated between cobalt when administered as the glutamate or the nitrate.

Summary. Cobalt (0.5 mg.), as cobalt glutamate and as cobalt nitrate, produces a polycythemia in young rats, when fed as a supplement to a whole milk (klim)-iron-copper diet. The animals receiving cobalt glutamate showed the same growth as the control groups, while that of the cobalt nitrate group was retarded.

7301 C

The Haptene-Protein Complex in Canine Anaphylaxis.

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Recent studies of specific allergic and immune reactions against artificial protein-crystalloid conjugates have suggested that the superficial crystalloids of a protein molecule are the essential units of specific antigenicity. If so, the whole theory of specific immunity must be rewritten in terms of sub-colloidal antigenic "determinants".

Most of these studies, however, are inconclusive. This is due in part to the high haptene-protein ratios almost invariably used. In many cases the conjugates were "carriers" of no less than 150 to 200 haptene "determinants".[1] Moreover, from the point of view of clinical medicine, both rabbit antiserum and guinea pig anaphylaxis distort the quantitative perspective. We have, therefore, repeated some of the classical tests, using haptene-protein complexes of relatively low ratios, with dogs as the experimental animals.

The proteins used in this work were horse serum (HS), cow serum (CS), dog serum (DS), egg white (EW) and crystallized excelsin (Ex). Conjugation was made with benzoyl (Bz) and benzene-sulfonyl (Su) radicals, the quantitative relationships being such as to give final products with an average of from 25 to 40 haptene groups per protein molecule. This represents an approximate 20% haptene saturation.

Dogs were sensitized by a single intracardial injection of 40 mg.

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[1] Kurtz, A. C., Sox, H. C., and Manwaring, W. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 138.

protein per kg. of body weight. This is the optimum sensitizing dose with HS. Anaphylactic tests were made 3 weeks later, the routine shock dose being 80 mg. protein by kg. of body weight injected intravenously. This is about 10 times the minimum shock dose with HS.

Fully 95% of all dogs become demonstrably hypersensitive after a single intracardial injection with HS and fully 75% with CS. In contrast with this, the percentage of successful sensitizations with BzHS, BzCS, SuHS, and SuCS was little more than 30%. Of the 37 dogs injected with these conjugates but 12 became demonstrably hypersensitive. BzEW, BzDS, and BzEx are apparently without sensitizing power for dogs.

Discarding 25 dogs which failed to become demonstrably hypersensitive following routine injection with haptene-conjugates, haptene and "carrier" cross-reactions were tested with 6 dogs. But 2 (33 1/3%) of these dogs gave definite (+ or ++) cross-reactions,

TABLE I.
Haptene and "Carrier" Cross-Reactions in Canine Anaphylaxis.
Severity of the anaphylactic reaction was judged from kymograph records: +, ++, +++ representing non-fatal shocks; ++++ recording lethal anaphylaxis.

Sensitizing antigen	Anaphylactic test		Retest with original antigen
	Antigen	Severity	
BzCS	BzDS	+	+++
BzHS	BzES	0	+++
BzHS	BzCS	±	0
BzHS	SuHS	++	0
BzHS	SuHS	0	++±
BzHS	SuHS	0	++

TABLE II.
"Carrier" Cross-Reactions with Native Proteins.

Sensitizing antigen	Anaphylactic test		Retest with original antigen
	Antigen	Severity	
HS	BzHS	+	0
HS	BzHS	+++	+++
HS	BzHS	0	++
HS	BzHS	0	++
HS	SuHS	0	+++
HS	SuHS	0	+++
HS	SuHS	+++	0
CS	BzCS	0	+
CS	BzCS	+	0
CS	BzCS	0	+++
CS	BzCS	+	++++

either with the haptene or "carrier" groups, in spite of the use of the decimultiple test dose. (Table I.)

Eleven "carrier" tests were made on dogs demonstrably hypersensitive to undenatured HS or CS (Table II). In but 5 (45%) of these dogs were "carrier" cross-reactions demonstrated with the decimultiple dose. But three (25%) of these "carrier" reactions demonstrably desensitized the dog to the homologous undenatured native protein.

The above data give a quite different quantitative perspective from that suggested by previous studies of haptene-saturated antigens and highly immunized or sensitized herbivorous animals.

7302 P

Differential Ovarian Responses After Injections of Follicle-Stimulating and Pregnancy Urine in Very Young Female Rats.*

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From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

It has been reported by Collip *et al.* that P.U. extract injections begun on the 6th day of life cause a thecal hypertrophy after 10 injections. In 5 litters of rats (each composed of 4 treated and 1 control) we have injected, daily, an amount of extract of follicle-stimulating urine which will induce a marked response in hypophysectomized male and female rats. Injections continued from the 6th to the 11th day of life up to the 16th or 17th day did not induce any ovarian change so far as we have been able to determine. Neither ovaries nor uteri were increased in weight and the vaginae did not open. In a series autopsied on the 21st day of life there was an increased ovarian weight and a marked stimulation of the follicles, but no thecal hypertrophy. These results show a marked difference between the action of F.-S.U. and P.U. extracts. They also show that the gamete and granulosa must undergo some maturation change before they are capable of responding to the follicle-stimulating hormone.

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* Aided by a grant from the Committee for Research in Problems of Sex, The National Research Council.

7303 P

Gametokinetic Action of Extracts of Follicle-Stimulating Urine.*

P. E. SMITH, E. T. ENGLE, AND H. H. TYNDALE.

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Injections of extracts of follicle-stimulating urine† in hypophysectomized males induce a marked proliferation and a maturation of the germ cells but do not affect the interstitial tissue of the testes or the accessory genital organs. This action has been shown in a series of experiments in male rats which were hypophysectomized when immature. In part of the series (immediate treatment) injections were begun at the time of hypophysectomy, in the other series (postponed treatments) a post-operative period of 7-40 days elapsed before beginning injections. Series of littermates were used. In the immediate treatments in each series there was a reference control (autopsied at the time of operation), usually an unoperated control (killed at the termination of the experiment) and one or more rats injected with F.-S.U. extract, P.U. extract,‡ and a mixture of these two extracts. In the postponed injections, 1 testis was ablated from each animal before beginning the various treatments.

Immediate Treatments. Five series, 19 hypophysectomized immature rats, age at operation 26-42 days. Duration of treatment 14-25 days. In every case the injection of F.-S.U. extract caused a marked proliferation of the germ cells. In certain of the tubules the secondary spermatocytes appear not to be normal, however, having vacuolated signet-ring nuclei. In the animals hypophysectomized at the younger ages, spermatids, and in the older ages, spermatozoa, were formed. Some of the latter were motile. In no case did the interstitial cells show hypertrophy and the accessory genital organs remained infantile in type.

In the combined injections (F.-S.U. + P.U.) sperm formation was increased over the F.-S.U. treatment alone. There was some, though not a marked response of the interstitial cells and the ac-

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* Aided by a grant from the Committee for Research in Problems of Sex, The National Research Council.

† The urine was collected from women past the menopause or after ovariectomy. An abbreviation of F.-S.U. is used for this follicle-stimulating urine which seems not inappropriate since pregnancy urine is commonly designated as P.U.

‡ Antuitrin S was used as the P.U. extract. Thanks are expressed to Dr. Kamm for this material.

cessories were greatly enlarged. In both types of treatment in the younger animals the testes show a marked increase in size.

Postponed Injections. Five series, 16 hypophysectomized immature rats. Both the F.-S.U. and the combined treatments caused a marked proliferation of the germ cells. The reparative effect on the tubules was somewhat greater in the combined treatments than when F.-S.U. only was injected. In both types of treatments as well as with P.U. extract injections the testes increase markedly in size.

The interstitial tissue of the testes remained atrophied in the F.-S.U. treatments and the accessory genital organs were of the hypophysectomized (castrate) type. In the combined injections there was an interstitial tissue response and the accessories enlarged. The interstitial cell response is still greater when P.U. extract, only, was injected. These observations offer strong evidence that the interstitial cells, only, secrete the male sex hormone.

These series of experiments show clearly that follicle-stimulating urinary extracts have an action only on the germ cells in males. There is no reparative effect on adrenals, thyroids, or body growth.

These findings are in harmony with those secured in hypophysectomized female rats. In them, treatment with F.-S.U. extract causes a maturation of ova and a proliferation of their genetic line of cells—the granulosa—which results in the formation of follicles with but little or no thecal response. P.U. extract, on the other hand, does not induce follicular growth but a marked hypertrophy of the interstitial tissue and theca and occasionally luteinization when the granulosa is sufficiently developed.

Both extracts cause oestrin secretion, whereas F.-S.U. extract does not stimulate secretion of male sex hormone.

7304 C

Experimental Production of Hypercalcemia in Human Beings by Means of Irradiated Ergosterol.

TOM D. SPIES AND RAMON F. HANZAL. (Introduced by J. T. Wearn.)

From the Institute of Pathology and the Department of Medicine, Western Reserve University, and the H. K. Cushing Laboratory of Experimental Medicine, Department of Medicine, Lakeside Hospital, Cleveland.

Since the work of Hess[1] Steenbock,[2] and others showing that by the administration of irradiated ergosterol in small doses it was possible to prevent or cure rickets, there has been much interest in the effect of this substance on all diseases concerned with the metabolism of calcium. A number of investigators[3-9] found that very large doses of irradiated ergosterol administered to experimental animals led to a rapid loss of weight and death. They observed an increase in the blood calcium and phosphorus during life and the post mortem examination showed that the arterial walls, heart muscle, stomach, lungs, kidneys, and other tissues contained deposits of calcium. The medical profession warned against the danger of excess vitamin D and viosterol was then restricted to an irradiated ergosterol content of 100 D. More recently it has been found that viosterol can be safely increased in potency with therapeutic benefit.[10]

It is certain from animal experimentation[5, 6] and from the recent observations of Crimm[12] on human beings that a hypercalcemia can be produced by means of irradiated ergosterol. It seemed probable, therefore, that this elevation in blood calcium and phosphorus might be controlled and used as a therapeutic aid in the treatment of disease. The present study shows that the oral administration to human beings of massive doses of irradiated ergosterol over short intervals of time produces an elevation of blood calcium and phosphorus without histopathological calcification of the tissues.

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[1] Hess, A. F., *J. A. M. A.*, 1910, **84**, 1925.

[2] Steenbock, H., and Nelson, M. T., *J. Biol. Chem.*, 1924, **62**, 209.

[3] Pfannenstiel, W., *Lancet*, 1928, **2**, 845.

[4] Kreitmair, H., and Moll, T., *Munchen med. Wchnschr.*, 1928, **75**, 637.

[5] Klein, I. J., *J. A. M. A.*, 1928, **92**, 621.

[6] Smith, M. I., and Elvove, E., *Pub. Health Rep.*, 1929, **44**, 1245.

[7] Shohl, A. T., Goldblatt, H., and Brown, H. B., *J. Clin. Invest.*, 1920, **8**, 505.

[8] Spies, T. D., and Glover, E. C., *Am. J. Path.*, 1930, **4**, 485.

[9] Spies, T. D., *Arch. Int. Med.*, 1932, **50**, 443.

[10] Report of the Council on Pharmacy and Chemistry, *J. A. M. A.*, 1930, **95**, 1021.

[11] Spies, T. D., unpublished data.

[12] Crimm, P. D., *Am. Rev. Tuberc.*, 1931, **23**, 576.

Four hopelessly diseased young adult patients were chosen for this experiment with the permission of their relatives. Three of the individuals had widespread miliary tuberculosis and the fourth metastatic carcinoma. Each patient was given the ordinary hospital diet and a mixture of 6 to 20 cc. of irradiated ergosterol* (10,000 D), grains XL sodium acid phosphate, and grains XL calcium lactate in one daily dose during the experimental period. In each instance the above mixture was administered during the last 9, 16, 16, and 25 days (the experimental period) of the patient's life. Frequent blood calcium[13] and phosphorus[14] determinations were made for a control period of a week before the beginning of the experiment, and were continued throughout the study. After the death of each patient, a complete autopsy was done and blocks of tissue from all organs were fixed in 10% formalin and imbedded in paraffin. Some of the sections were stained with hematoxylin and eosin and others were treated by the silver method of Von Kossa.

The 4 patients had a steadily progressive downward course. The

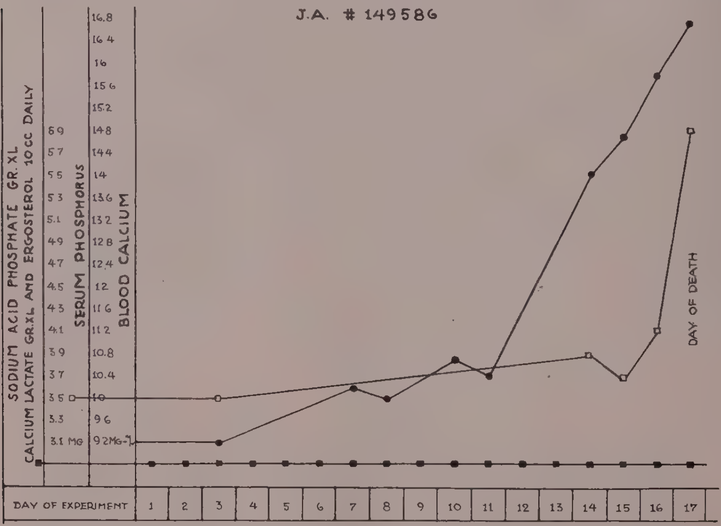

FIG. 1.

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* This ergosterol was furnished through the courtesy of Dr. C. E. Bills of Mead Johnson and Company.

[13] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **48**, 223.

[14] Benedict, S. R., and Theis, R. C., *J. Biol. Chem.*, 1924, **61**, 63.

blood calcium and phosphorus rose as high as 16.6 mg. per hundred cc. respectively (Chart I), but as far as could be ascertained the hypercalcemia produced no symptoms. Post mortem examination showed no changes attributable to the elevation of calcium or phosphorus. The lesions of each case appeared the same as similar lesions seen in other patients who had died of tuberculosis or cancer. Histological examination of the sections from each patient revealed the characteristic changes found in tuberculosis or carcinoma but no evidence of calcium deposits.

It has been shown that a marked elevation of the calcium and phosphorus content in the blood of 4 human beings, under the conditions of this experiment, does not cause calcification of the tissues. Many investigators[7–10] have found that excessive doses of irradiated ergosterol caused elevation of the calcium and phosphorus in the blood of laboratory animals with subsequent calcification of the aorta, heart, kidneys, lungs, stomach, spleen, and other organs. This study, showing that the deposition of calcium salts does not occur in any of the tissues until after a severe hypercalcemia has been produced, supports unpublished observations noted in laboratory animals. It is likely that an amount of irradiated ergosterol, many times the therapeutic dose for rickets, might harm adults by producing extreme elevation of calcium and phosphorus in the blood and calcification in various tissues of the body. The potential danger from its excessive administration, however, should not prevent the use of it in therapy so long as its effect is controlled by frequent phosphorus and calcium determinations.

At the present time therapeutic intravenous injections of calcium are given for many conditions such as abdominal pain, blood dyscrasia, lead poisoning, etc. Since any intravenous administration is somewhat dangerous and since that of calcium is particularly likely to cause necrosis of the surrounding tissues and nerves,[18] it seems that the oral administration of somewhat larger doses of irradiated ergosterol may be safer and more efficacious. The elevation of calcium and phosphorus should be controlled and arbitrarily should not exceed more than 12 and 4 mg. per 100 cc. respectively. This method offers a higher and a more lasting intravascular concentration of calcium with less likelihood of complications following its administration than the present method of injecting calcium salts intravenously.

Summary and Conclusions. 1. A marked elevation of the blood calcium and phosphorus occurred in 4 adult human beings without pathological calcification of the tissues. 2. This evidence suggests that the carefully controlled oral administration of viosterol produces a higher and more lasting concentration of blood calcium with fewer dangers of local complication than the present method of injecting calcium salts intravenously.

7305 P

Action of Pregnancy Urine Extract (Follutein) on the External Genitalia of Female Guinea Pigs.*

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Injections of Follutein† into sexually immature female guinea pigs produce an enlargement of the clitoris. Animals treated over a long period show a considerable enlargement. The clitoris takes the form of a typical penis which protrudes when the surrounding tissues are subjected to pressure. The prepuce, the corpus cavernosum, and the glans are well differentiated. The size of the enlarged clitoris is considerably smaller than the size of a male penis, in animals of corresponding age. Adult female guinea pigs treated with Follutein respond likewise, although the growth of the clitoris is less pronounced than in the young females.

Experiments were made on a group of 48 animals. After the administration of 3-5 cc. of Follutein over a period of about 10 days, changes can be observed in the clitoris. These become very pronounced after a more prolonged administration over 4-6 weeks.

The results do not imply necessarily a specific action. The histological examination of an experimentally enlarged penis-like clitoris reveals a general enlargement of the organ. No definite specific effect is noticeable in any of its tissues.

A marked increase in circulation and pronounced swelling in the external genital region appear after treatment. Vascular changes as a result of injections of pregnancy urine have been noticed previously by Papanicolaou.[1] He advanced the hypothesis that the luteinizing effect on the ovaries, as well as the stimulating action

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* This work has been aided by the Committee for Research on Sex Problems of the National Research Council.

† Follutein was supplied by E. R. Squibb and Sons, New York.

[1] Papanicolaou, G., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 807.

upon other glands, might be interpreted as being primarily due to vascular changes.

Preliminary work on ovariectomized animals shows that the growth potentiality of the clitoris almost disappears after complete removal of the ovaries. There is, however, an indication of a circulatory stimulation. This suggests that at least 2 separate factors are involved. One factor induces congestion and swelling and acts primarily on the clitoris itself; the other factor induces growth and acts secondarily through the stimulation of the ovaries.

The transformation of the clitoris into a penis is not necessarily a specific reaction. Since the clitoris has fundamentally the same morphological structure as the penis, it possesses the potentiality to develop into a penis-like organ, when subjected to a growth stimulus.

Injections of Follutein into male guinea pigs have a comparable effect on the penis. Swelling of the external genital region and increased circulation and swelling of the penis are induced. When the injections are administered into very young males, before the full growth of the penis, pronounced growth effect is noticeable. This effect disappears, apparently, through castration, comparable to the effect in the female after ovariectomy. In the adult normal male guinea pig the growth effect is not apparent but the congestion effect is present.

7306 P

Formation of Agglutinins Within Lymph Nodes.

PHILIP D. McMASTER AND STEPHEN S. HUDACK.

From the Rockefeller Institute for Medical Research, New York City.

Experiments were planned to determine whether or not lymph nodes form antibodies against antigens injected into the peripheral lymphatic capillaries.

On 2 successive days killed cultures of *B. paratyphosus-B* were intradermally injected into the right ear of a large series of mice. The cervical lymph nodes on the right side became much enlarged while those on the left side appeared normal. At varying intervals up to 12 days after the last injection groups of 10 animals were etherized, bled for serum and the lymph nodes which drain the lymphatics of the ears excised. The right nodes were pooled in one group, the left in another. They were ground with sand, extracted with Ringer's solution and the extracts and serum titrated for agglutinins by 3 methods, the microscopic, the macroscopic and the centrifugation-macroscopic method of Gates.[1]

On the second,third and fifth days after the last injection no agglutinins could be demonstrated in the node extracts or sera; but on the seventh day agglutinins were strongly positive in the extract from the nodes on the injected (right) side at a dilution of 1 in 120. They were questionably positive in the serum at a dilution of 1 in 30. The agglutinin titre was increased in both materials as procured on succeeding days, remaining slightly stronger in the node extract. At length, on the twelfth day, antibodies appeared in the extract from the nodes on the uninjected side.

The results suggested a possible formation of antibody by the nodes. But those on the injected side were inflamed, while those on the other side were not. It was conceivable that the inflamed nodes had merely taken up agglutinin formed elsewhere in the body and brought to them on the blood. There was yet another possibility due to the dilatation of the blood vessels in the ear caused by the injections. As is well known, dilated vessels are more permeable than normal; and it seemed possible that the agglutinins found in the lymph nodes had escaped from the blood vessels into the tissues of the ear and were drained to the nodes by the lymphatics or, further, that agglutinins possibly formed in the ear had been similarly conveyed there. To test these alternatives other experiments were done. Killed cultures of *B. paratyphosus-B* were injected intradermally in the right ears and Schick test toxin in the left. This procedure caused inflammation of the nodes on both sides and dilatation of the blood vessels in both ears. A week later, agglutinins for *B. paratyphosus-B* were found at a dilution of 1 to 240 in the extract from the nodes on the side injected with the organisms. In the serum they were present at a dilution of 1 to 60. None was found in the node extract of the opposite side.

In another type of experiment, the same antigens were injected in the same way into the ears but once only. Three hours later the ears injected with paratyphoid bacterin were cut off. Nine days later, in the node extract from the paratyphoid injected side, agglutinins were found at a 1 to 120 dilution, but none from the extract of the nodes on the other side. The serum gave faintly positive agglutinin reaction at 1 in 60.

Finally 2 agglutinin forming antigens, namely, killed cultures of

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[1] Gates, F. L., *J. Exp. Med.*, 1922, **35**, 63.

B. enteritides and of *B. prodigiosus* were injected intradermally in the right and left ears respectively of a series of mice. The injections were made on 2 successive days in some experiments, while in others 5 to 8 injections were given at intervals up to 2 weeks. From one to 3 weeks after the first injection extracts of the pooled right nodes and left nodes were titrated for agglutinins for both of the antigens as was also the pooled serum. Cross agglutination tests were negative. From 4 to 16 times as much agglutinin for *B. enteritides* was found in the node extract from the side injected with that bacterin, as in the extract from the other side; and from 3 to 10 times as much as in the serum. The findings for prodigiosus agglutinin were similar, that is to say, the titre for prodigiosus agglutinin was found far higher in the extract from the nodes of the side injected with *B. prodigiosus,* much less was present in the serum, and still less, or as much as in the serum in the node extract from the opposite side. The lymph glands on the 2 sides were invariably inflamed to about the same extent. The experiment was repeated 4 times with similar results.

The longer the time interval between injection of antigen and examination for antibody, up to 3 weeks, the greater was the concentration of the latter in both serum and nodes. In the experiments in which a single antigen was used, the concentration of antibody in serum and nodes became about equal within three weeks or less but the titre of the serum never exceeded that of the glands.

The findings will be fully reported elsewhere.

7307 P

Agglutinin Formation Within the Lymph Nodes of Resistant and Susceptible Mice.

STEPHEN S. HUDACK AND PHILIP D. McMASTER.

From the Rockefeller Institute for Medical Research, New York City.

An accompanying paper has reported the formation of agglutinins within lymph nodes following intradermal injection of killed cultures of bacteria in the ears of mice. Earlier work from this laboratory has shown such intradermal injections to be largely intralymphatic. After injections of killed bacteria into the ear of an animal a cervical lymph node draining the lymphatic capillaries enlarges and a week later agglutinins in high concentration can be found within it. At this time the blood shows a lower concentration of agglutinin and the lymph nodes elsewhere in the body yield no demonstrable antibody. It is evident that the procedure engenders, in the early days of the immunizing process, a high concentration of agglutinin in a localized region.

We have experimented to find whether the lymph nodes of strains of generally susceptible and resistant mice developed by Webster[1, 2, 3, 4] differ in the ability to form agglutinins to certain bacteria to which they are susceptible and resistant respectively, and to other organisms against which their relative resistance is unknown.

Approximately 200 mice of each of the strains mentioned were obtained through the courtesy of Doctor Webster. A similar number of stock white mice of the ordinary laboratory mixed strain were employed for comparison. In each experiment groups of 12-20 mice from each strain were injected in the skin of both ears with killed cultures of various agglutinin forming bacteria, *B. enteritides, B. paratyphosus-B* or *B. prodigiosus,* using but one antigen in each experiment. The cervical lymph nodes draining the ears were removed after varying intervals up to 10 days following the last injection, and the animals were bled for serum. The individual nodes from the mice of each group were pooled and extracted and the titre of the extract was compared with that of the serum, and with that of the node extracts and sera of the other groups.

The concentration of specific agglutinin in the lymph node extracts of the generally susceptible strain of animals was found to be definitely greater than that of the resistant ones. So, too, with the serum titre. The control group yielded findings which were intermediate or else like those in the generally resistant strain of animals.

The mice of the strain manifesting a general lack of resistance seemed capable of forming more specific agglutinin than those of the resistant strain. For example, in a typical experiment the susceptible, resistant and mixed strains of mice received 5 intradermal injections of 0.02 cc. of a suspension of killed *B. enteritides* during a period of 15 days and 21 days after the first injection agglutination tests of the cervical lymph node extracts and sera were done, as described in the preceding paper.

The lymph node extracts of the susceptible, resistant and mixed strain mice showed positive agglutination at dilutions of 2048, 512,

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[1] Webster, L. T., *Harvey Lectures,* 1932, **27**, 154.

[2] Webster, L. T., *J. Exp. Med.,* 1930, **52**, 931.

[3] Webster, L. T., *ibid.,* 1933, **57**, 793.

[4] Webster, L. T., *ibid.,* 1933, **57**, 819.

and 1024, respectively, and the sera at dilutions of 1024, 512, and 512, respectively. The findings will be published *in extenso* elsewhere.

7308 P

Experimental Hemosiderosis.*

VALY MENKIN.

From the Department of Pathology, Harvard Medical School.

Many pathologists have assumed that hemosiderin is a product in the partial degradation of hemoglobin. It is recognized mostly as phagocyted material of a yellowish-brown color within mononuclear phagocytes, liver cells, or cells of the kidney tubules. It yields a Prussian blue reaction when tissues containing it are treated with acidified potassium ferrocyanide. The substance is therefore iron-containing in distinction to hematoidin. Brown[1] pointed out a number of years ago that hemosiderin-like products can be obtained experimentally by the injection of hematin derivatives. These substances he termed *hemosideroid*, for he found that they were soluble in hydrogen peroxide or in potassium hydrate. True hemosiderin was found to be insoluble when treated with these agents. Sprunt[2] and more recently Whipple[3] have pointed out the possibility that hemosiderin may be the result of a change in the fundamental pigment metabolism of the organism rather than a product in the partial degradation of hemoglobin.

For the past few years the writer[4, 5, 6, 7] has demonstrated, in studies on inflammation and tuberculosis, that following intravenous injections of ferric chloride, the iron salt accumulates at the site of an acutely inflamed area; and in tuberculous rabbits, within the tubercles themselves, when the injections are given repeatedly. Concomitantly with this accumulation the life span of experimental rab-

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* This investigation was aided by grants from the William W. Wellington Memorial Research Fund and by the DeLamar Mobile Research Fund.

[1] Brown, W. H., *J. Exp. Med.*, 1911, **14**, 612.

[2] Sprunt, T. P., *Arch. Int. Med.*, 1911, **8**, 75.

[3] Whipple, G. H., and Bradford, W. L., *Am. J. Dis. Child.*, 1932, **44**, 336.

[4] Menkin, V., *J. Exp. Med.*, 1930, **51**, 879

[5] Menkin, V., and Menkin, M. F., *J. Exp. Med.*, 1931, **53**, 919.

[6] Menkin, V., *J. Exp. Med.*, 1932, **55**, 101.

[7] Menkin, V., *Am. J. Med. Sci.*, 1933, **185**, 40.

bits is prolonged and the course of their disease is protracted. The ferric chloride when injected in the circulation forms a ferric proteinate precipitate which, in the concentration of the salt employed (0.25%), immediately redissolves. The writer[6] pointed out that hemosiderin-like granules were frequently found in the spleen, liver, bone marrow, and at times in the kidney tubules of rabbits repeatedly injected with ferric chloride.

A summary of further studies on the production and distribution of hemosiderin is here presented. Twenty-eight tuberculous rabbits that had received no ferric chloride were studied for the distribution of hemosiderin in the spleen, liver, and bone marrow. The spleens of 9 animals in this group showed small amounts of a brownish iron-staining material within mononuclear phagocytes. The spleen of the remaining 19 rabbits showed absolutely no sign of hemosiderosis. There was no trace of hemosiderin in either the liver or bone marrow in any of the rabbits. On the other hand 28 rabbits that had received repeated intravenous injections of 0.25% ferric chloride for a number of weeks yielded some interesting information in regard to the production and distribution of material indistinguishable from hemosiderin. In the spleens of 26 rabbits out of a total of 27 studied, massive deposits of this pigment were found. Mononuclear cells both in sinuses and reticular cords of the spleen were loaded with this yellowish-brown material. Upon treatment with potassium ferrocyanide the pigment yielded a strong positive reaction for iron. When the microchemical tests with 3% and 30% hydrogen peroxide or with diluted potassium hydrate solution, as described by Brown, were applied, the pigment failed to dissolve, acting thus in a manner identical to true hemosiderin. The pigment was found deposited in all sections of bone marrow and in about half of the livers studied. In the latter the pigment was located for the most part within the Kupffer cells, while in the former, yellow, iron-containing granules were seen in the reticular cells of the bone marrow. Identical results were obtained when ferric chloride was injected in non-tuberculous rabbits. Accompanying the hemosiderin deposits in the spleen, variable degrees of fibrosis replacing in part the peripheral portions of the Malpighian follicles were noted.

These observations reveal the fact that repeated injections of diluted ferric chloride solution in the circulating blood are followed by the deposition, largely within the cells of the reticulo-endothelial system, of a pigment which by known criteria is indistinguishable from true hemosiderin. The conclusion is warranted that a typical state of hemosiderosis has been experimentally reproduced by repeated administration of an iron salt. Rous and his collaborators[8] obtained the deposition of hemosiderin by the injections of hemoglobin. Evidences on hand, accumulated in collaboration with Mr. S. Talmadge, show that repeated injections of ferric chloride do not have any effect on the hemoglobin or red cell counts of rabbits that might thus indirectly account for the hemosiderosis obtained. Further studies on this phase will be reported subsequently. The observations obtained indicate that hemosiderin is not solely a product in the degradation of hemoglobin but may result from a release in the body fluids of iron (presumably in combination with proteins) from cells in general. This material, when ultimately phagocyted, appears in the familiar form of hemosiderin.

7309 C

Inversion of the P Wave in the Third Lead of Electrocardiograms with a Large Q-3 Wave.

CHARLES SHOOKHOFF AND ALBERT H. DOUGLAS. (Introduced by Harry Gold.)

From the Cardiological Division of the "B" Medical Service, the Jewish Hospital of Brooklyn.

In the course of a study of electrocardiograms which show a large Q-3 deflection, we noted what appeared to be a relative frequency of inversion of the P deflection in the third lead of these tracings. This appeared to be consistent with the finding of Carr, Hamilton and Palmer[1] that inversion of P-3 is occasionally associated with the production of a large Q-3 wave in electrocardiograms taken from pregnant women.

We undertook to determine the frequency with which an inverted P-3 wave is found in an indifferent series of electrocardiographic tracings and in a series of electrocardiograms which contain a large Q-3 wave.

No attempt was made in this study to exclude or separate electrocardiograms showing auricular fibrillation, auricular flutter, paroxysmal auricular tachycardia or nodal rhythm. In tracings where extrasystoles were present these were ignored—the P-waves associated with the normal sinus rhythm alone were examined. No attention was paid to diphasic P-waves or to isoelectric P-intervals. Only when the P-wave was wholly inverted was it included in this study. The large Q-3 waves were selected in accordance with Pardee's criteria[2]: 1—the Q was at least 25% as large as the largest deflection of Q-R-S; 2—there was no S-wave; 3—waves of the M and W types were omitted.

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[8] Rous, P., and Oliver, J., *J. Exp. Med.*, 1918, **28**, 629.

[1] Carr, F. B., Hamilton, B. E., and Palmer, R. S., *Am. Heart J.*, 1933, **8**, 519.

Analysis of 2500 consecutive electrocardiograms taken at the Jewish Hospital of Brooklyn revealed 73 with an inverted P-3 wave (3%); one with an inverted P-1 wave (0.04%); and one with an inverted P-2 wave (0.04%). Of the group of 73 with an inverted P-3 wave, 50 (68%) showed deviation of the ventricular electrical axis to the left; 20 (27%) showed normal direction of the ventricular electrical axis, and 3 (5%) showed deviation of the ventricular electrical axis to the right. Eight of the group of 73 showed a large Q-3 wave. The single record with an inverted P-1 showed deviation of the ventricular electrical axis to the left. The single record with an inverted P-2 showed inversion of P-3 as well, and a normal direction of the ventricular electrical axis.

One hundred and twenty-nine electrocardiograms were found in

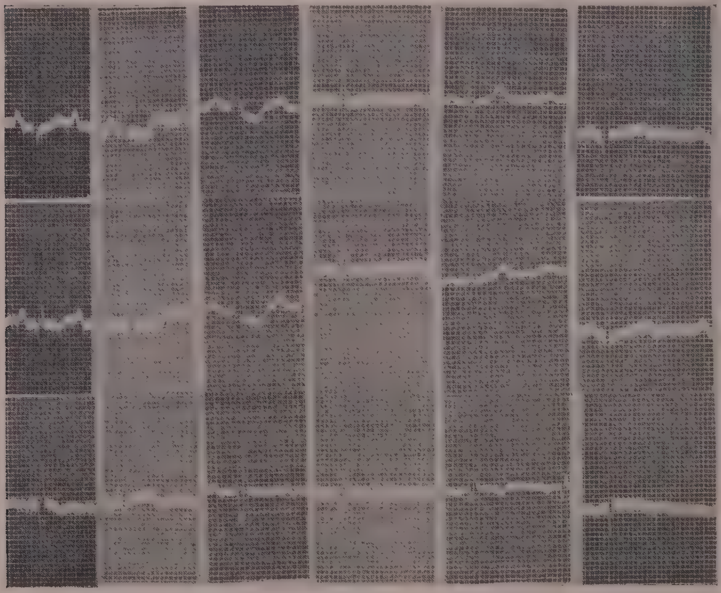

FIG. 1.
Examples of large Qs and inverted P-waves in the third lead.

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[2] Pardee, H. E. B., *Arch. Int. Med.*, 1930, **46**, 470.

another series of 2500 consecutive records (not the same list reported above) which showed a large Q-3 wave according to Pardee's criteria (5%). Twenty-eight of the series of 129 records showed inversion of the P-wave in Lead III (22%). Inversion of P in Leads I and II was not found in this series.

The results given indicate that inversion of P-1 or of P-2 is relatively rare while inversion of P-3 is not uncommon. There is a definite association between inversion of P-3 and inversion of Q-R-S-3—about two-thirds of the records with an inverted P-3 showed deviation of the ventricular electrical axis to the left. This association suggests the possibility that the inverted P-3 might bear the same relation to a rotation of the auricular electrical axis (in a counter-clockwise direction) that the inverted Q-R-S-3 bears to a similar rotation of the ventricular electrical axis. Early investigations on inverted P-waves by Lewis,[3] Ritchie,[4] V. Hoesslin,[5] Einthoven, Fahr and de Waart[6] suggested that intrinsic auricular causes, such as change in the position of the pacemaker in the auricle (as, for example, that which follows vagal stimulation) could cause inversion of the P-wave. The last named authors suggested also rotation of the auricle as a whole as another possible cause for inversion of the P-wave. That this was possible was shown by Cohn[7] when he rotated chest leads in a clockwise manner, which simulated rotation of the heart to the left and upward, and produced inversion of P-3, of Q-R-S-3 and of T-3. Master and Oppenheimer[8] came to a similar conclusion after an electrocardiographic study of 97 obese patients in which left axis deviation of Q-R-S and inversion or flattening of P-3 appeared in three-quarters of the records. Their view that these changes were caused by elevation of the diaphragm and rotation of the heart appeared to be confirmed by the fact that 13 of 15 patients who subsequently lost weight showed a change of P-3 from flat or inverted to upright. Bland and White[9] made a study of complete inversion of Lead III in human electrocardiograms and they concluded that the inversion of P-3 as well as of Q-R-S-3 and T-3 in these tracings was caused by a rotation of the heart as a whole to the left.

Data presented in this paper show a definitely increased frequency of inverted P-3 waves in electrocardiograms that also show a large Q-3 deflection—22% as compared with 3% in an indifferent series. In the light of the discussion given above, this finding could be explained by assumption of a counter-clockwise rotation of the auricular electrical axis in these cases. However, we recently presented evidence[10] for a belief that the large Q-3 wave is usually representative of a rotation of the ventricular electrical axis *about a longitudinal axis* to the left. In the light of this work it appears possible that the increased frequency of inverted P-3 waves in these records may partly be the result of a similar rotation of the auricular electrical axis about a longitudinal axis to the left. We have already mentioned the work of Carr, Hamilton and Palmer,[1] who found occasional inversion of P-3 associated with the production of a large Q-3 in electrocardiograms from pregnant women. Elevation of the diaphragm, as in pregnancy, can probably cause rotation of the heart as a whole in either a vertical or a horizontal plane or both, with the production of a large Q-3 or a deep S-3, associated with an inverted P-3.

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[3] Lewis, T., *Heart*, 1910, **11**, 2.

[4] Ritchie, W. F., *Quart. J. Med.*, 1912, **6**, 47.

[5] von Hoesslin, H., *Deutsch. Arch. f. Klin. Med.*, 1914, **113**, 537.

[6] Einthoven, Fahr, and deWaart, *Arch. f. d. ges. Physiol.*, 1913, **100**, 275.

[7] Cohn, A. E., Heart, 1921-1922, **9**, 311.

[8] Master, A. M., and Oppenheimer, E. T., *J. A. M. A.*, 1929, **92**, 652.

[9] Bland, E. F., and White, P. D., *Am. Heart J.*, 1931, **6**, 333.

Summary. 1. Three percent of 2500 consecutive electrocardiograms showed an inverted P-3 wave. 2. Of 73 electrocardiograms with an inverted P-3, 68% showed left axis deviation; 27% no deviation of the ventricular electrical axis; and 5% right axis deviation. 3. Of 129 electrocardiograms with a large Q-3 (taken from another series of 2500 records) 22% showed an inverted P-3 wave. 4. The association between inversion of P-3 and inversion of Q-R-S-3 can be explained by the assumption that counter-clockwise rotation of the ventricular electrical axis is not infrequently associated with counter-clockwise rotation of the auricular electrical axis. 5. The increased frequency of inverted P-3 waves in electrocardiograms with a large Q-3 may be due to rotation of both ventricular and auricular electrical axes about a longitudinal axis to the left. In all probability the electrical axes of the heart frequently shift with relation to both vertical and horizontal planes rather than in one alone. 6. Elevation of the diaphragm, as in pregnancy, can probably cause rotation of the heart as a whole in either a vertical or a horizontal plane or both, with the production of a large Q-3 or a deep S-3, associated with an inverted P-3.

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[10] Shookhoff, C., and Douglas, A. H., Proc. Soc. Exp. Biol. and Med. 1933, **31**, 209.

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Progressive Muscular Anemia in the Heart of a Dog.*

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Evidence has already been presented[1] to show that interruption of the blood supply to a given ventricular muscle band will produce a characteristic and constant alteration in the electrocardiogram.

TABLE I.†

Muscle	Characteristic Electrocardiogram	
S.B.S.	T very neg. in I, II, III	R-T depressed in I S-T elevated in II, III
D.B.S.	T positive in I, II, III (Fig. 1a)	R-T very elev. in I, II, III
S.S.S.	T slightly neg. in I, II, III	R-T elevated in I, II, III
D.S.S.	T_1 neg., T_3 pos. (Fig. 1b)	R-T " " I S-T very depressed in II, III

† Note correction of misprint in original publication.

The present report is concerned with consecutive electrocardiograms from a single dog in which the coronary blood supply to muscle after muscle was obstructed. The technique was as previously described, allowing half hour intervals between successive ligatures. Ligatures were placed near the apex on the vessels supplying: the internal portions of the Superficial Sino- and Bulbo-spiral muscles, the deep Sino-spiral (both right and left portions), the deep Bulbo-spiral, the Septum (4 ties 1 cm. apart from below upwards on the anterior descending coronary) and one on the branch from the left circumflex coronary to the upper posterior septum. Finally, the whole left circumflex branch of the left coronary was ligated. At this time the only adequate blood supply was to a small area on the anterior surface of the right ventricle, to the right portion of the scroll muscles and to the bundle of His.

As soon as a vessel is ligated the area involved ceases to contract, assumes a dusky color and bulges slightly. The electrocardiogram indicated that when the ligations were made serially in one animal, each characteristic modification reported previously was again introduced as a typical addition to or a subtraction from the previous tracing.

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[1] Robb, J. S., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 311.

The amplitude of the records became progressively greater as the lesions progressed. When only this small area of the right ventricle could be seen to beat there was a high R in Lead I. This experiment was later repeated with similar results.

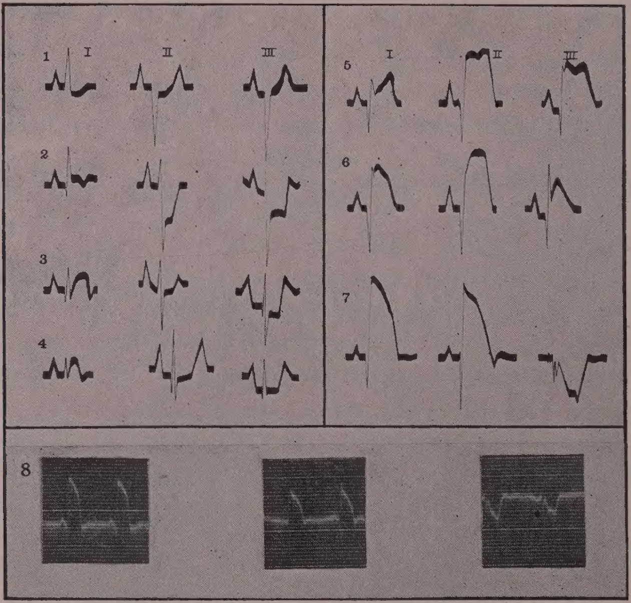

Fig. 1.

Original tracings taken with standard technique. Numbers 1 to 7 redrawn to scale.

1. Operated control, indirect leads.
2. Initial lesion of deep sino-spiral.
3. Characteristic alteration for lesion of the superficial bulbo-spiral, R-T elevation of I lowered, S-T much elevated in II, III as compared to No. 2.
4. Lesion of superficial sino-spiral, further elevation of R-T segment. Note lessened S in all leads.
5. Anemia of the deep bulbo-spiral elevated the R-T segments in all leads.
6. Septal involvement, note the exaggeration of Q and the increased downstroke of R in III.
7. Left circumflex coronary ligated. Only a small portion of the right ventricle beating. Note the tall R in lead I.
8. Original tracing from which No. 7 was redrawn.

In these experiments injury to the septum resulted in the development or in the exaggeration of the Q wave. Very large areas could be damaged without fibrillation appearing provided only the blood supply to the bundle of His remained intact.

Conclusions. 1. A characteristic electrocardiographic change is present for each ventricular muscle, regardless of the order of involvement, when one after another is deprived of its blood supply.

2. The type of complex called a Laevogram by Lewis[2] and a Dextrogram by Barker, Macleod and Alexander[3] is obtained when only a small area of the right ventricle is visibly contracting.

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[2] Lewis, T., 1925, Mechanism and Graphic Registration of the Heart Beat, Shaw and Sons, London.

[3] Barker, Macleod, and Alexander, *Am. Heart*, 1930, **5**, 720.